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Effect of Methylene Blue on Tumors.*

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This paper deals with the results of experiments obtained with rat carcinoma-sarcoma No. 256/94 A from the Crocker Institute inoculated subcutaneously into a selected strain of rats, and with mice as described below. In the rats, the per cent of non-takes was 6.5. The spontaneous regressions, *i. e.*, cases in which tumors were never larger than about 1 mm. in size and disappeared after a few days, were about 10%. These were not used in making computations in either set of controls or dye-treated animals. Only those tumors which reached a size larger than this and then disappeared were considered "regressions".

Previous experiments which will be described in the full report were unsatisfactory because of the high percentage of non-takes and spontaneous regressions. This paper will deal only with a group of animals in which this difficulty has been largely eliminated.

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* Aided by grants from the Cancer Fund established at the University of California Medical School by a friend, by the Christine Breon Fund for Medical Research, and by the National Research Council of Washington, D. C.

There were 51 control animals and 87 in the treated group. Treatment, which was started when the tumor had reached a size of about 0.5 cm., consisted of daily injections into the tumor of 0.01% aqueous solution of methylene blue (Merck's medicinal). This dye was found to be the most effective of a group of oxidation-reduction indicators varying in redox-potential according to Clark's scale. The amount injected varied with the size of the tumor, which was difficult to gauge accurately, but was usually about 1/10 cc. for each mm. length of tumor; however, not more than 2 cc. was used for the large tumors to avoid possible toxic effects of the dye on the animal.

The average length of life of those animals having tumors which did not regress was 49.4 days in the controls and 45.2 in the dye treated. Those animals whose tumors regressed lived indefinitely and are therefore not included in the calculation of the above averages. The average size of the tumors of those animals which died was 6.25 cm. in the controls and 5.4 cm. in the dye-treated. The tumors of the dye-treated animals usually did not become so large as those of the controls.

The per cent regressions in the controls was 5.5; that in the dye-treated was 44. The regressing tumors usually became necrotic, sloughing off and gradually shrinking with scab formation, and finally disappearing.

Experiments with mice were not so satisfactory as those on rats just described, because the dye had to be given in smaller concentrations to avoid toxic effects on the animals. The most satisfactory group was a strain containing a mammary tumor from Memorial Hospital. These tumors never regressed in the controls but eventually killed the animal. However, in a group of 14 dye-treated animals, there were 4 regressions, while 10 animals bred at the same time and used as controls for this set gave no regressions.

The injection of methylene blue into tumors of rats and mice appears to favor regression of the tumors. This may result from one or both of the following actions; first, poising of the tissue at a redox-potential unfavorable to tumor survival, and second, the catalytic effect of methylene blue acting as a donor of activated oxygen to the tissues. Since this second effect depends upon cyclic oxidation and reduction of the dye, it can only occur when the redox-potential of the tissues lies at an appropriate level, namely, that at which the dye itself tends to poise the tissue. The two effects are therefore interdependent. It seems important in this connection that other dyes, above or below methylene blue in the redox scale, are relatively ineffective. The importance of the amount and type of oxidation as a factor in tumor physiology is apparent from the work of Warburg on glycolytic metabolism of tumors. The present work does not, however, afford sufficient basis for excluding the possibility of other factors being operative, nor for further elaboration of hypotheses as to the interrelationship between the effects of methylene blue, redox potentials, type and amount of oxidation, and tumor physiology.

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Effect of Cholesterol Feeding under Varying Conditions upon Lipids of Rat Livers.

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Our interest has been drawn during the past few years to the question of the effect of various dietary deficiencies or excesses upon the deposition of cholesterol and cholesterol ester in tissues; manifested pathologically as fatty livers, arteriosclerotic arteries and gall stones. The following is a preliminary report of the first of a series of studies of tissues of rats fed cholesterol with diets of known but varying fat content, vitamin intake, etc.

The litters of rats used were cut to 8, 4 males and 4 females, shortly after birth. At 21 days of age, 2 males and 2 females from each litter were placed upon the diets containing cholesterol, while the littermate controls were placed upon the same diets without the cholesterol. After a feeding period of approximately 60 days the rats from both groups were killed by cutting the spinal cord at the base of the brain. The tissues were separated as quickly as possible and samples weighed for determination of moisture and of lipid content and in some cases for histological examination. The samples for lipid determinations were ground with sand and extracted to exhaustion with alcohol-ether.

The present report is limited to the composition of the livers of 2 groups of 16 animals each fed 1% cholesterol with basal diets consisting of 20% baked and extracted casein, 4% Osborne-Mendel salt mixture, 4% agar. In the first series this was supplemented with 15% Crisco and 47% starch, while in the second series 10% Crisco and 62% starch were used. For the experimental groups the cholesterol was dissolved in the melted Crisco and thoroughly mixed with the rest of the diet, so that cholesterol intake was strictly proportional to food intake.

Northwestern yeast was used as a source of vitamin B complex for the first 40 days of each experiment after which a fully adequate amount of Harris yeast extract was substituted. We had planned to use carotene as a source of vitamin A but the only preparation available proved unsatisfactory. Hence we were compelled to use cod liver oil, which was given at the rate of one drop per day. Otherwise the control diets were sufficiently low in sterol that the alcohol ether solubles material from 1 gm. diet gave no color with the Liebermann Burchard reagent. Yeast and cod liver oil were therefore the chief sources of sterol in the control diets.

The food intake of the animals was such that the total cholesterol fed per day was, at a very rough approximation, 1 mg. per gram rat.

The sterol fed animals showed not only a marked increase in the fatty acid content of the livers, but also an almost phenomenal increase in sterol as ester. The liver fatty acids for the control rats on the diets containing 15% Crisco were approximately 12% as determined by oxidation, for the cholesterol fed animals, 23%. The corresponding figures for sterol as ester were 0.19 and 7.25% respectively.

Free sterol was very nearly the same for the 2 groups, *i. e.*, about 0.25%.

Sterol ester percentages for the animals given only 10% fat in the diet were only slightly lower. In this group, however, the livers of the female animals fed cholesterol seemed to enlarge less than those of the males. Hence, while the actual weight of sterol stored was slightly greater in the males, the percentage concentration was higher in the females. Fatty acids were actually higher in the livers of the females. The number of animals used so far is probably too small to justify conclusions as to the influence of sex on storage of lipid in the liver, but the results are at least suggestive of the necessity for more work. Unfortunately in our series of determinations on the 15% fat intake each of our samples was made up of the livers from 2 males and 2 females mixed together for extraction.

In one group of animals from each series the vitamin B complex (as Harris yeast extract) was removed from the diet 10 days before killing. The livers from the cholesterol-fed animals in these groups showed a distinct increase in moisture content and a marked decrease in both fatty acid and sterol as ester. Littermates fed vitamin B complex + cholesterol at the 10% fat level showed 16.6% fatty acid and 6.0% cholesterol as ester for the males, 23.2% fatty acid and 6.6% cholesterol as ester for females. Those deprived of the yeast extract showed 11.9% fatty acid for males, 13.9% for females and 4.5% for males and 2.5% for females cholesterol as ester, respectively. However the figures for the animals receiving no cholesterol showed about the same proportionate decrease in fatty acids, 8.5% to 5.4% for males and 10.5 to 5.6% for females. Cholesterol as ester in both was negligible in amount.

Further data will be necessary to determine whether this is a result of lowered food absorption alone, but some as yet incomplete studies indicate that the effect is specific for vitamin deficiency.

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On the State of Calcium and Phosphate of the Blood.

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The report by Smith and Sternberger[1] that in many of their ultrafiltration experiments the sum of the ultrafiltrate and concentrate calcium fell very considerably short of the serum calcium and that the difference was due to an adsorption of calcium by the collodion membranes, led us to undertake an investigation of the phenomenon. This loss is important in relation to the differentiation of the blood calcium into a diffusible and non-diffusible fraction by procedures such as the one of Greenberg and Gunther.[2]

It was found that the loss from serum with only normal amounts of calcium is negligible and only when the serum calcium is very considerably augmented, either by *"in vitro"* or *"in vivo"* measures, does the amount of calcium taken up by the membrane become of importance. The significance of diffusible and non-diffusible calcium determined under the usual circumstances on fresh blood serum is in no way vitiated by this finding.

This is borne out by the experiment given in Table I, which is representative of our results. The table shows the changes produced

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[1] Smith, R. G., and Sternberger, H. R., *J. Biol. Chem.*, 1932, **96**, 245.

[2] Greenberg, D. M., and Gunther, L., *J. Biol. Chem.*, 1929, **85**, 491.

TABLE I.

Effect of $CaCl_2$ Injection on Calcium and Phosphate Diffusibility.*

Dog St. Wt. 20 kilos. Injected arterially with 20 ml. 0.25 M $CaCl_2$.

Time hr.	Serum Ca	Diffusible Ca	Serum Ca Calc.	Loss	Serum Phosphate	Diffusible Phosphate	Loss
Control	11.15	7.25	11.45	0	4.60	4.80	0
0.25	16.20	8.90	13.50	2.70	4.30	3.10	1.20
1.3	13.75	8.00	11.65	2.40	5.70	4.60	1.10
3	12.45	6.65	12.30	0.15	5.10	5.05	0.05
5.5	12.05	6.25	12.25	0	4.40	4.15	0.25

* All blood chemistry values in mg. per 100 ml.

in the blood of a dog injected arterially with 0.5 gm. $CaCl_2$. In the original control blood sample and after the calcium has dropped back to the range of the usual serum values, the calculated serum content from the ultrafiltrate and the concentrate analysis agrees as well with the determined values as could be expected. In the samples with high calcium the loss is considerable.

It was found, moreover, that under these conditions not only the calcium but phosphate also is taken up by the membranes, and what is of greater significance, *the phosphate which normally is completely diffusible becomes in very considerable part non-diffusible.* This effect can be more strikingly shown *"in vitro"* by augmenting serum with calcium. An experiment of this kind in which beef serum has been augmented with calcium chloride is shown in Table II. With increasing calcium content the diffusibility of the phosphate becomes progressively less.

TABLE II.

Beef Serum Augmented with $CaCl_2$.

No.	Total Ca	Diffusible Ca	Total Ca Calc.	Loss	Total Phosphate	Diffusible Phosphate	Loss
1	9.30	3.95	8.35	0.95	7.70	6.35	0.65
2	14.10	5.10	10.50	3.60	7.70	5.20	2.50
3	17.90	6.60	12.55	5.35	7.70	4.20	3.50
4	22.25	7.80	18.30	5.95	7.70	3.15	4.55
5	26.60	10.70	23.95	2.65	7.70	2.65	5.05

Our experiments have led us to the hypothesis that the phenomenon is the result of the formation in sera high in calcium of a colloidal material composed of calcium, phosphate and probably carbonate which is stabilized and prevented from precipitating by the protective action of the serum proteins. We believe that this hypothetical colloidal substance is what is taken up by the collodion membranes. Actually, the action is not an adsorption in the usual sense but instead is the result of an impregnation of the pores of the membrane produced by the ultrafiltration. Collodion membranes cut up into shreds and shaken with calcium augmented serum did not take up any calcium or phosphate whatsoever.

Another reason for believing that it is a colloidal form of calcium and phosphate which is impregnated on the membrane and not calcium and phosphate ion as such is given by the experimental findings with magnesium. When the magnesium in the blood is augmented by injection there is no such loss on the filtration membrane as is found with calcium. A typical experiment is given in Table III. The agreement between the serum magnesium and the values calculated from the ultrafiltrate and concentrate magnesium here is gratifying. Because of the close chemical relationship of calcium and magnesium, if adsorption of the cations were involved, both elements would be expected to show a similar behavior.

TABLE III.
Effect of $MgSO_4$ Injection on Magnesium Diffusibility.
Dog Fl. Wt. 14 kilos. Injected subcutaneously with 10 cc. of 20% $MgSO_4$.

Time, hr.	Total Mg.	Diffusible Mg.	Total Mg. calculated
Control	2.15	1.90	2.15
0.25	5.10	4.10	4.50
1.3	5.25	4.05	5.20
4.5	2.35	2.05	2.20

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Effect of Infra-Red Light on Subsequent Fertilization of the Eggs of Certain Marine Invertebrates.*

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A study has been made of the effect of near infra-red radiation on the subsequent fertilization of the eggs of some marine invertebrates. The species studied were 2 sea urchins *(Strongylocentrotus purpuratus* and *S. franciscanus)* and a worm *(Urechis caupo)*. Light from a Mazda standard projection lamp (gas filled, 30 volts, 30 amperes, with the tungsten filament radiating at about 3200°K) was passed through a slit 1x18 mm., 10 cm. from the filament, and through a monochromator consisting of 2 glass collimating lenses having a maximum thickness of 6 mm. each, and two 60° quartz prisms. An adjustable outlet slit was so arranged as to pass that portion of the spectrum lying within the approximate limits 0.8-1.2μ as determined by calculation from the known dispersion. The wave length of maximum energy was determined by the use of a small vacuum thermocouple and found to lie at about 0.8-0.9μ, as would be expected from the nature of the source. Rough calculation suggests that the energy flux in this region was probably of the order of magnitude of 0.1 cal. cm.$^{-2}$ min.$^{-1}$

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* This work was founded upon and includes a portion of the work done by one of us (S.C.B.) with the assistance of M. H. Simmers under grants from the Committee on the Effects of Radiation on Living Organisms (National Research Council).

Unfertilized eggs in a single layer were irradiated for periods of 15 to 45 minutes in special thin glass cells, controls in similar cells being placed 2 cm. away on the top of the monochromator. The experiments were done in darkness, the source being in a separate room, so that the controls received only that small amount of infra-red and scattered visible light which passed through the monochromator and was reflected by the walls of the dark room. After irradiation the eggs were fertilized and further development watched under ordinary artificial illumination. For purposes of this article the term fertilization is used to designate the formation of fertilization membranes. The eggs of *Urechis* showed quantitatively identical effects upon this process and upon the disappearance of the germinal vesicle and subsequent cleavage.

In all cases the proportion of eggs fertilized decreased. The table shows the results of the experiments under the following column heads in order: Species; experiment number; duration of irradiation; n_2 and n_1, the total numbers of eggs in the control and irra-

TABLE I.

The effects of infra-red light on subsequent fertilization of eggs. See text for details.

Species	Exp. No.	Irradiation	n_2	n_1	p_2	p_1	p_2-p_1	$\epsilon_{p_2-p_1}$	$\frac{p_2-p_1}{p_2}\cdot 100$	$\frac{p_2-p_1}{\epsilon_{p_2-p_1}}$
		min.								
Strongylocentrotus purpuratus	I	30	1341	1263	.541	.321	—.221	±.019	—40.7	11.6
”	II	30	1439	1414	.051	.011	—.040	±.020	—78.4	2.0
”	III	30	951	923	.358	.046	—.312	±.053	—87.1	5.9
”	IV	45	1123	1292	.300	.089	—.211	±.016	—70.3	13.2
”	V	45	1265	928	.596	.186	—.410	±.019	—68.7	21.6
S. franciscanus	VI	30	461	570	.269	.219	—.050	±.027	—18.5	1.8
Urechis caupo	VII	15	509	628	.203	.081	—.122	±.015	—60.0	8.1
” ”	VIII	30	1376	1211	.307	.190	—.117	±.016	—36.3	7.3
” ”	IX	45	279	298	.570	.416	—.154	±.041	—27.0	3.8

diated samples; p_2 and p_1, the corresponding fractions successfully fertilized; $p_2 - p_1$, the decrease in the fraction fertilized; $\epsilon_{p_2 - p_1}$, the standard error of this difference for simple samples; $\frac{(p_2 - p_1)}{p_2}.100$, the per cent decrease in successful fertilization, and $\frac{p_2 - p_1}{\epsilon_{p_2 - p_1}}$, the ratio of the decrease to its standard error. The last two columns, therefore, show the magnitude of the effect and its statistical significance. Since all the experiments agree in showing a decrease in fertilization, they may all be considered significant; either experiments II or VI alone would not be significant. In the first case unavoidable ageing of sperm and eggs before use greatly lowered the percentage fertilized and probably accounts for the poor result. Exp. VI represents our only experiment with *S. franciscanus,* and in it relatively few eggs were used. The relatively inconclusive result in this case may be due to either chance error of small samples, or more resistant eggs. The former seems more probable.

The decrease in fertilization is probably not due to heating; thermocouples showed at no time during irradiation a rise of more than 0.04°C. in the temperature of the sea water in which the eggs were lying. Previous studies by Simmers, using a Pt-Fe microthermocouple as described by Whitaker,[1] have shown that the temperature within these eggs does not during irradiation rise above that of the surrounding medium enough to affect a measuring system sensitive to 0.02°C. The total temperature difference between control and irradiated eggs was certainly not as much as 0.06°C.

Even this slight rise in temperature might be suspected to coincide with some evaporation of sea water from the irradiated sample. But since repeated renewal of this sea water during irradiation did not affect the outcome, evaporation was not a factor.

There appears to remain only one possibility: that infra-red light of wave length 0.8-1.2μ affects photochemically some constituent of these eggs and thus inhibits subsequent fertilization. We know of no previous experiments in which biological effects attributed to infra-red light may not have been produced indirectly as a result of heating. Unless some factor which we have not considered is operative, our results are, therefore, the first demonstration of a photochemical effect of infra-red light on living cells.

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[1] **Whitaker, D. M., *Science*, 1929, 70, 263.**

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Observations on the Intranasal Route of Infection in Experimental Poliomyelitis.*

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It is generally recognized that while experimental poliomyelitis may be induced with regularity in monkeys inoculated with a potent virus by the intracerebral route, infection results much less frequently when the virus is instilled into the nasal passages. Since the natural infection in man occurs chiefly, if not entirely, by the nasal route, it is sometimes desirable, especially in certain experimental studies, to imitate closely the natural mode of infection. To be really serviceable any method employed must produce infection with considerable regularity. While the results of different investigators with the intranasal route of inoculation have been but meagerly reported, the general experience seems to have been that while infection by the intranasal route is possible, it is seldom realized in more than one-half of the monkeys so inoculated. At times less than a third of the animals develop the disease. In exceptional instances only have somewhat better results been reported. Rhoads[1] and Weyer, Park and Banzhaf[2] have recently reported quite uniform results following 3 or more intranasal instillations of virus, *administered on successive days.* The total number of monkeys employed by these investigators is not specifically stated, nor do they describe in detail the procedure. The reasons for their more uniform results are therefore not apparent. Having ourselves worked with what were evidently the same strains of poliomyelitis virus, we are unable to attribute their exceptional results altogether to the employment of viruses of unusual virulence. It has become obvious to the writers that while the virulence of a virus, as well as the number of instillations, are undoubtedly of great importance in establishing infection by the intranasal route, factors other than these apparently also influence the results, including possibly such factors as the general anatomical and physiological status of the mucous membranes; the properties of the nasal secretions; the reaction (pH) of the aqueous

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* These studies were supported by the Mrs. Mary Hooper Somers Medical Research Fund.

[1] Rhoads, C. P., *J. Exp. Med.*, 1931, **53**, 115.

[2] Weyer, E. R., Park, W. H., and Banzhaf, *J. Exp. Med.*, 1931, **53**, 553.

vehicle of the virus, etc. Amoss and Taylor[3] reported that washings of nasopharyngeal mucous membranes of normal persons may at times possess the power to inactivate the virus of poliomyelitis. This ability to inactivate the virus was not constantly exhibited by secretions obtained at different times from the same individual. Flexner and Amoss[4] later revealed that the nasal mucosa of individual normal monkeys may also possess virus inactivating properties. The exact nature of the substance responsible for the inactivation was not determined, but appeared to be distinct from the specific immune substances which make their appearance in the blood of poliomyelitis convalescent animals and man. It is conceivable, therefore, that the condition of the nasal mucous membranes and of the nasal secretions of individual monkeys may influence to some extent the possibility of establishing infection by the intranasal route.

Needing a dependable extracerebral route of infection in some studies which we wished to complete, we made a more comprehensive inquiry into the possibilities of the intranasal route of infection. Working with the so-called Aycock strain, we had earlier found that ordinary nasal instillations, even though repeated on several successive days, failed to give more than 50% infection; nasal tampons saturated with virus, plus ordinary virus instillations, proved even less effective. Several monkeys inoculated by submucous injections of virus failed to develop poliomyelitis. Following this another strain of poliomyelitis virus (MV) was obtained from Dr. Simon Flexner and our studies were carried on with this strain. The results proved distinctly superior to those we had realized with the Aycock strain. Of a total of 9 monkeys given 3 intranasal instillations of this virus, all instillations being made on the same day, 7 (77%) developed the disease. Since a higher incidence of infection than this was desired, we endeavored to determine the effect of washing thoroughly the nasal passages immediately before each intranasal instillation of virus, employing a M/15 phosphate solution (primary potassium phosphate adjusted to pH 5.0 with secondary sodium phosphate), the nasal passages being thoroughly irrigated before each virus instillation with about 25 cc. of this solution. Since we have applied these preliminary nasal washes the number of infections seem to have been definitely increased. Up to the present out of a total of 42 monkeys inoculated according to this procedure 40 (95%) have developed poliomyelitis. The average incubation period in this series of animals was about 8 days. The individual virus instillations, 0.5 cc. in amount each, were made at 3 hour intervals on the same day, a 10% virus suspension in physiological saline (pH 6.3) being employed. Just what effect the *acid* rather than alkaline virus suspension had in giving us a higher percentage of infection, was not determined. The virus suspensions were allowed to stand at refrigerator temperature (2°C.) from one to 4 days before the intranasal instillations were made. They were in each instance prepared from sections of cords and medullas derived from 4 or 5 monkeys; the sections being *pooled* and ground thoroughly in a motor driven mortar for at least one hour.

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[3] Amoss, H. L., and Taylor, E., *J. Exp. Med.*, 1917, **25**, 507.

[4] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1920, **31**, 123.

To determine whether the results realized were due merely to the washing itself, or to both the mechanical effect of washing and the reaction (pH) of the solution used, preliminary intranasal washings were carried out with phosphate buffer solutions of different pH values.† Of 3 monkeys receiving intranasal washes with M/15 phosphate buffer of pH 7 only 1 developed poliomyelitis; of 2 washed with M/15 phosphate buffer of pH 8.3 neither developed the disease. Of 4 washed with Sorensen's borate buffer, pH 8.9, 2 developed the typical disease; one developed an abortive form of poliomyelitis 17 days after inoculation and one failed to develop the disease.

Summarizing our observations, we may state that our results indicate that the incidence of infection by the intranasal route may be definitely increased by preliminary intranasal washes with acid buffer solution. Alkaline solutions appear less effective. Our results also show that with a virus of high infectivity, 3 intranasal instillations, preceded by intranasal washes, will result in infection in about 95% of the animals so treated. These virus instillations may all be made on the same day, thus enabling one to date the infection with more accuracy than is true when the instillations are made on successive days over a period of 3 days to a week, as is the common practice.

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† We have observed that the nasal secretions recovered by washing the nasal passages of monkeys may shift the physiological saline from pH 6.5 to pH 7.15, a shift from the acid to the alkaline side.

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Alleged "Reflex" Occular Immunity.*

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Following repeated injection of *B. typhosus* vaccine into an anterior chamber of the rabbit eye, Shamburow[1] found the specific *B. typhosus* agglutinin titer of the aqueous humor of that eye increased to as much as 8 times the synchronous agglutinin titer of the blood stream. The non-vaccinated eye often showed 2 to 3 times the synchronous serum titer. He believes his data prove: (1) a local synthesis of specific agglutinin in the vaccinated eye, and (2) a neurological reflex, causing a similar local antibody synthesis in the non-vaccinated eye.

In our attempts to confirm his data, 8 half-grown rabbits were repeatedly injected in one anterior chamber with heat-killed or living *B. typhosus,* doses and time intervals being the same as that used by the Russian investigator. Forty-eight parallel titrations of the resulting antiserums and immune aqueous humors are summarized in Table I, each recorded titer being the mathematical average of the readings from 8 rabbits.

TABLE I.

Samples withdrawn immediately before	*B. typhosus* agglutinin titer Blood Serum	 Vaccinated Eye	 Non-vaccinated Eye
1st *B. typhosus* injection	1:10	0	0
2nd injection (7th day)	1:1380	1:352	1:1.2
3rd injection (14th day)	1:1710	1:388	1:1.2
4th injection (21st day)	1:2840	1:400	1:1.2
28th day	1:2840	1:500	1:5
35th day	1:1160	1:500	1:5
42nd day	1:940	1:225	1:5
49th day	1:640	1:125	1:5(?)
56th day	1:445	1:42	1:1.2

In no individual rabbit of our series did the agglutinin titer of the aqueous humor of the vaccinated eye exceed one-half of the synchronous serum titer, nor did the titer in the non-vaccinated eye even exceed 1% of that of the blood stream. From these data we see no reason to assume either a local or a "reflex" synthesis of specific agglutinins in the occular tissues.

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* Supported in part by the Rockefeller Fluid Research Fund of Stanford Medical School.

[1] Shamburow, D. A., *Z. f. Hyg. u. Infektionkr.*, 1932, **114**, 456.

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Comparative Studies of Gonad-Stimulating Hormones.* II. Influence of Length of Period of Administration of Certain Extracts.

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The importance of the "time factor" in studying the responses elicited in immature rats by gonad-stimulating hormones has been previously mentioned.[1, 2] A significant difference in the effects produced by a pregnancy blood extract from those by a sheep anterior pituitary extract has been noted when the *same total dose* of each preparation is administered over various periods of time.

The method of preparing the extracts has been described.[1, 2, 3] The injections were begun in rats 20 to 23 days of age, and the extracts were made up in various dilutions so that each animal received the required total dose when 0.25 cc. was administered twice daily for the duration of the experiment. A total of over 200 rats was used in studying the various aspects of this problem.

In one series, 23 animals were given 2.5 cc. of a blood extract equivalent to 5.75 cc. of a mixture of several specimens obtained from pregnant women. Five rats injected for 5 days showed an average increase over controls of 200% in ovarian weight and 309% in uterine weight (ave. body weight 34 gm., ovaries .036 gm., uterus .136 gm.). When the same dose was given to 5 rats in 10 days, the average increase in ovarian weight was 450% and in uterine weight 565% (ave. body weight 48 gm., ovaries .066 gm., uterus .199 gm.). In 15 days the ovaries of 5 rats gave an average increase of 387% in weight and the uteri of 570 % (ave. body weight 47 gm., ovaries .058 gm., uterus .207 gm.). The same total dose was given in 20 days to 8 rats, which showed an average gain in ovarian weight of 525% and in uterine weight of 615% (ave. body weight 56 gm., ovaries .083 gm., uterus .240 gm.).

In another series, 23 rats received a total dose of 2.5 cc. of an acid

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* Supported by a grant from the Rockefeller Fluid Research Fund of Stanford University School of Medicine. The sheep anterior pituitary glands were supplied by Messrs. Parke, Davis & Company, through the courtesy of Dr. E. A. Sharp.

[1] Fluhmann, C. F., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 149.

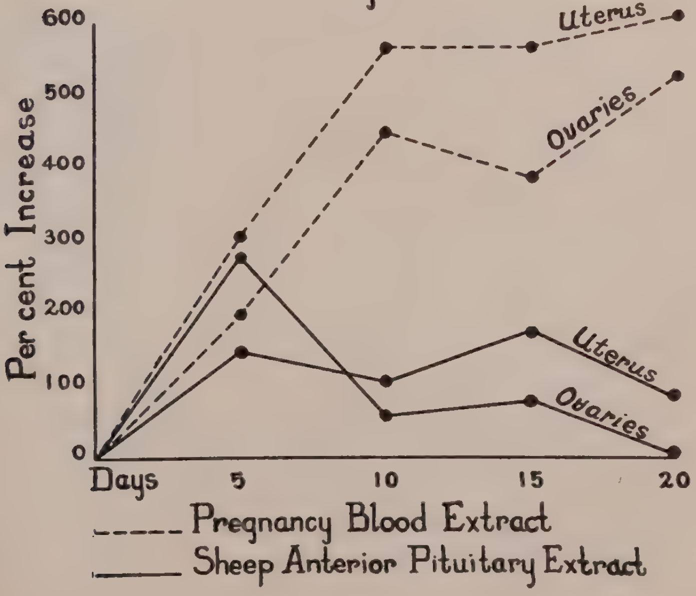
[2] Fluhmann, C. F., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 881.

[3] Fluhmann, C. F., *Endocrinology*, in press.

extract of sheep anterior lobe made up so that 1.0 cc. of extract was equivalent to 500 mg. of the dried pituitary powder. The injection of this dose to 6 rats resulted in 5 days in an average increase in ovarian weight of 280% and in uterine weight of 152% (ave. body weight 32 gm., ovaries .048 gm., uterus .078 gm.). In 10 days, the average increase in weight of the ovaries of 5 rats was only 60% and of the uterus 111% (ave. body weight 35 gm., ovaries .019 gm., uterus .072 gm.). In 15 days, 6 rats gave an average increase in ovarian weight of 100% and in uterine weight of 175% (ave. body weight 41 gm., ovaries .022 gm., uterus .075 gm.). When the same total dose was given over a 20-day period to 6 rats, no increase in ovarian weight over uninjected controls was observed and the average increase in uterine weight was only 85% (ave. body weight 47 gm., ovaries .012 gm., uterus .055 gm.).

It is thus seen that in the case of the pregnancy blood extract there is a tendency to produce greater increases in ovarian and uterine weight as the period of administration is prolonged (Fig. 1). In a series of experiments comparing the results of 5 and 10-day periods of injection, it was found that this phenomenon is also characteristic of untreated human pregnancy blood serum, Antuitrin-S (Parke,

Fig. 1



Davis and Company), Collip's A-P-L extract of human placenta (Eli Lilly and Company), and Follutein (E. R. Squibb and Sons).

On the other hand, with sheep anterior pituitary extracts there is a marked diminution in the effect on ovarian weight when the period of administration is prolonged, and this finding also applies to acid extracts made from human hypophyseal tissue. An exception must be made for some instances when doses of anterior lobe extracts are employed which produce more than 1000% increase in ovarian weight in 5 days, as a greater response may then be obtained in 10 days. It would seem that in this case the ovaries are unable to respond completely to the excessive stimulus in the short period of 5 days.

Summary. The administration of a known total dose of an ovary-stimulating pregnancy blood extract over periods of 10, 15, or 20 days produced a much greater increase in ovarian and uterine weights of immature rats than when the same total dose was given in 5 days. The opposite result was obtained with acid extracts of sheep anterior pituitary glands, as the injection of a known dose in 5 days produced a greater increase in ovarian weight than when the administration of the same total dose was spread out over periods of 10, 15, or 20 days.

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6776

Studies of the Etiology of Influenza.

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For many years the etiology of influenza has been the subject of an interesting and continuous discussion. A number of microscopically visible organisms have been assigned a certain causative relationship to this disease. The most important of these microorganisms is *H. influenzae,* the significance of whose relationship to influenza was first brought out by Pfeiffer. This organism has continued to hold a prominent position on the stage of debate concerning the disease. Another question that has led to much discussion is the degree of uniformity of nature of the pathological and clinical entity described as influenza. Interest in this regard has largely centered upon the identity or non-identity of the so-called pandemic and inter-pandemic forms of the disease. Clinically, examples of the disease in pandemic and in inter-pandemic periods may resemble each other so closely as to be indistinguishable. Whether they are the same condition from an etiologic standpoint can not, however, as yet be answered.

In recent years, with increasing knowledge of filterable viruses, this group of micro-organisms has been prominently considered in discussions of the etiology of influenza. Some evidence[1-5] has already been brought out tending to implicate them in the disease and it is with this aspect of influenza that the present work is concerned.

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[1] Dujarric de la Riviere, M. R., *Comp. rend. Acad. des Sci.,* 1918, **167**, 606.

[2] Selter, H., *Deut. Med. Woch.,* 1918, **44**, 932.

[3] Nicolle, C., and Lebailly, C., *Ann. de l'inst. Past.,* 1919, **33**, 395.

[4] Yamanouchi, Sakakami, Iwushima, *Lancet,* 1919, **1**, 971.

[5] Long, P. H., Bliss, E. A., Carpenter, H. M., *J. Am. Med. Assn.,* 1931, **97**, 1122.

During an outbreak of influenza of the inter-pandemic type in the winter of 1930, an effort was made to communicate the disease to chimpanzees by means of bacteria-free filtrates of nasopharyngeal washings obtained from patients with typical attacks of the disease. The washings were obtained during the first 36 hours of the attack, passed rapidly through Seitz filters and inoculated intranasally in chimpanzees. In all, 8 animals were so treated. In no instance did any of the apes manifest either local respiratory symptoms or recognizable constitutional reaction of any kind. All of the chimpanzees inoculated were recovering or had recently recovered from typical attacks of the common cold so that susceptibility may have been low.

Shortly after this 2 human volunteers were placed in strict quarantine for a period of 4 days. At the expiration of this time nasopharyngeal washings were obtained from an individual with an attack of influenza of the prevailing type. The important symptoms and signs of this patient were: Temperature 101°F. by mouth, general malaise, cough, and nasal obstruction, leucocytes 7,300. The patient was confined to bed for 4 days.

Volunteer 25 was inoculated intranasally with 6 cc. of a Berkefeld N filtrate of the above washings. The filtrate was negative both aerobically and anaerobically for bacterial growth. Within 24 hours of inoculation this volunteer developed nasal obstruction, sneezing, slight nasal discharge and slight headache. Later there was red throat and a productive cough. There was no fever and no constitutional reaction. By the third day the symptoms had abated considerably and the volunteer was discharged from the hospital. He appeared to have had a mild but definite common cold. One day after discharge the symptoms reappeared in more severe form. The subject was readmitted to the hospital with acute pharyngitis, fever of 102°F., marked constitutional reaction, cough and muco-purulent sputum. The manifestations lasted for about 4 days.

Volunteer 26 was inoculated with the same material and in a manner similar to Volunteer 25. This volunteer experienced no symptoms of respiratory infection either during his stay or after discharge from the hospital.

During an outbreak of influenza in February, 1932, a culture was made, in chick embryo medium, of filtered bacteria-free nasopharyngeal washings from an acute case of influenza. The technique employed was that used by us in the cultivation of the virus of the common cold.[6] The patient's symptoms were typical except for a mild cystitis. There were two slight chills, the temperature reached 103°F., moderate cyanosis was present, mild conjunctivitis, running nose and slight sore throat. The leucocytes were at first 7,000 and later dropped to 4,840. The duration of the fever was 4 days.

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[6] Dochez, A. R., Mills, Katherine C., and Kneeland, Yale, Jr., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 513.

This culture F. was carried in chick embryo medium, transferring every 3 or 4 days for 19 generations, a period of about 10 weeks. The F 19 generation was inoculated intranasally into Volunteers 55 and 56, under strict isolation. Volunteer 55 developed slight malaise and headache within 24 hours of the time of inoculation. Later there developed sneezing; nasal discharge; productive cough; red, sore throat; and moderate constitutional reaction. There was no fever. The white blood cells were 9,500. This individual experienced the symptoms of an acute common cold of marked severity.

Volunteer 56 developed symptoms synchronously with those of Volunteer 55. There was moderate constitutional reaction and very marked manifestations of respiratory tract inflammation. There was no fever and the white blood count was 10,650.

Both of these individuals experienced the symptomatology of the common cold. The attacks were of marked severity and acute signs of infection lasted for 7 days. Three weeks later the same 2 volunteers were re-inoculated with the F 25 generation of the culture in order to test for the presence of immunity. Both individuals again experienced attacks resembling the common cold, but of less severity than the preceding attacks.

Following these inoculations 5 more volunteers were inoculated and of these 4 experienced symptoms of a mild common cold and one, no reaction whatever. During the summer of 1932 the culture was transferred to London and in collaboration with C. H. Andrewes of the National Institute for Medical Research, 17 volunteers were inoculated. Of these 15 experienced no symptoms whatever. Two developed infections resembling the common cold, one having a moderate constitutional reaction and a temperature of 99.2°F. Inasmuch as isolation was not practiced and colds were prevalent at the time, reliable conclusions could not be drawn from the positive instances of infection.

The culture was brought back to the United States and has since been carried continuously in chick embryo medium. Generations F 57, F 65, F 80 and F 83 have been inoculated into human volunteers. Generation F 57 was inoculated into 3 individuals and induced mild symptoms of upper respiratory inflammation of the type of the common cold. Generation F 65 was inoculated also into 3 subjects, all of whom developed symptoms of upper respiratory infection within 36 hours after inoculation. The symptomatology in these individuals showed a change from that previously observed in the volunteers inoculated with cultures of typical common cold virus. The upper respiratory symptoms were relatively slight and consisted of sneezing, moderate nasal discharge and cough and mild sore throat. On the other hand, the constitutional reaction consisting of headaches, anorexia, general malaise with aching pains in the back and legs and a rise in temperature up to 1°F. was more pronounced than we have as yet observed in any individuals experimentally infected with tissue medium cultures from examples of acute upper respiratory infection. Generations F 80 and F 83 were each inoculated into 2 volunteers. These 4 individuals suffered from acute upper respiratory infections of the type described above, manifesting slight signs of respiratory tract irritation, constitutional reaction of the type just described and very slight fever. The duration of the symptoms ranged from 3 to 5 days.

Chimpanzees have been relatively insusceptible to experimental infection with the tissue culture virus of the common cold and with that cultivated from influenza. However, one of these animals has been successfully inoculated with Culture F 63. The animal had experienced no respiratory infection for 5 months. It was placed under strict quarantine and after a fore-period of observation was inoculated intra-nasally with generation F 63. Twenty-four hours later nasal obstruction and discharge appeared. For 4 days thereafter there were manifest definite but mild symptoms of the common cold. There was no fever and no prostration. Another animal inoculated at the same time with the same material exhibited no symptoms.

Culture F, isolated from the naso-pharynx of an individual suffering from clinical influenza, has over a period of 11 months produced experimental upper respiratory infections in a series of human volunteers. The character of the infection has been of 2 types, the first resembling the common cold with conspicuous signs of irritation of the respiratory tract, little constitutional reaction and no fever; the second somewhat different with slight signs of respiratory inflammation, moderate constitutional reaction and slight fever, never above 1°F.

In January, 1933, a second chick embryo medium culture was made of filtered bacteria-free, naso-pharyngeal washings from an acute case of influenza. There was at this time a rather widespread outbreak of typical clinical influenza. This patient exhibited the usual symptoms, the temperature at the time the culture was made was 101.6° F., and the leucocytes were 5,800. The manifestations of respiratory irritation were slight and there were no complications.

This culture was carried in chick embryo medium, using the technique previously described, for 2 months when generation T 18 was inoculated intranasally into 2 human volunteers under strict isolation. Both of these individuals became infected and exhibited a similar symptomatology. Signs of infection appeared at the end of 24 hours and were still present at the time of discharge from quarantine 7 days later. The symptoms consisted of nasal obstruction, sneezing, marked nasal discharge, cough with the production of muco-purulent sputum, and redness and slight edema of the throat. The constitutional reaction was very slight and there was no rise in temperature. These 2 experimental infections resembled severe examples of the common cold and were very much like the first series of infections produced by inoculation of culture F 19 described above. Control inoculation of the same individuals, before experimental infection, with filtered naso-pharyngeal washings from a normal human being and with incubated sterile tissue culture medium have been negative except for moderate signs of temporary irritation.

By employing the technique used by us for the cultivation of the virus of the common cold it has been possible to propagate in tissue culture medium a filtrable agent from 2 typical clinical examples of influenza. Human volunteers have been repeatedly infected by intranasal inoculations with this cultivated agent, in one instance after the agent had been carried for 11 months in the culture medium and when it had reached the eighty-third generation. The symptomatology of the experimental infections in general has resembled that of the common cold. The respiratory infections induced have been somewhat more severe in character than those which have developed after inoculations with the cultivated virus of the common cold. Following inoculation with generations F 65, F 80, and F 83 the infected volunteers showed, in addition to the ordinary symptoms of the common cold, moderate constitutional reaction and slight fever. We believe that these experiments indicate that a filtrable agent has been cultivated from patients suffering from influenza. We have not as yet been able to distinguish this agent from that of the common cold. Because of the short duration of immunity in human beings, sometimes not more than 3 weeks, it has not been possible to perform experiments designed to bring out the immunological relationship between the agent described and that of the common cold.

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A Simply Prepared Broth for Producing Hemolytic Streptococcal Hematoxin (Streptolysin).*

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Todd[1] has demonstrated recently that the sera of patients suffering from various hemolytic streptococcal infections contain antibodies that neutralize streptococcal hematoxin, and he, collaborating with Coburn[1, 2] has shown that these antibodies are almost constantly present in abnormal concentrations in the serum of patients suffering from rheumatic fever. Obviously a new technique has been developed for the study of infections of this type. Todd's[3] original medium for the production of streptolysin consisted of horse meat infusion, peptone and yeast extract, which after mixing was sterilized by filtering, first through a coarse earth filter and then through Chamberland "F" candles. Later Todd and Hewitt[4] found that a more suitable medium could be prepared by substituting for the yeast extract the following substances: sodium bicarbonate as a buffer, inorganic phosphates and glucose as growth stimulating materials and sodium chloride to supply suitable isotonicity.

We have had little success with the broth containing yeast extract, but have obtained a satisfactory yield of streptolysin from the buffered horse meat broth, and a still better yield when fresh beef heart was substituted for the horse meat. The tedious and time-consuming filtration of the broth, through 2 different filters, makes the production of this medium very difficult unless special apparatus is available. It is possible, on the other hand, to prepare a medium highly satisfactory for the production of streptolysin without filtering the broth. This is done by sterilizing the mixture of meat infusion and peptone in the Arnold apparatus, and subsequently adding the solution of salts and glucose which has been sterilized previously by filtering through a Berkefeld candle. The medium, prepared by the appended method, is usually clear, and not infrequently contains a slight granular sediment. The disadvantage of contamination while adding the salts and glucose is far outbalanced by the constant yield of streptolysin and the heavy growth of bacteria. A 15-hour growth of both streptococci and pneumococci has given nearly 4 times the volume of bacterial sediment that was obtained from the same quantity of good beef heart infusion broth. This medium is, therefore, very useful for obtaining heavy bacterial growths, and this effects a saving in both time and material. This broth passes through a Chamberland filter much more readily after streptococci have grown in it than before inoculation. The beef heart infusion 2% proteose peptone, reinforced with 0.2% dextrose, has furnished practically as good a hemolysin-yielding medium, but the amount of streptococci obtained from a unit of this broth has been smaller than that obtained from the medium described below. The detailed procedure for the manufacture of the broth and the production of streptolysin is as follows:

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* The generic term *hematoxin* is used for bacterially produced substances that lyse red blood cells, and the specific term *streptolysin* for such substances elaborated by hemolytic streptococci.

[1] Todd, E. W., *Brit. J. Exp. Path.*, 1932, **13**, 248.

[2] Coburn, A. F., and Pauli, R. H., *J. Exp. Med.*, 1932, **56**, 651.

[3] Todd, E. W., *J. Exp. Med.*, 1932, **55**, 267.

[4] Todd, E. W., and Hewitt, L. F., *J. Path. and Bact.*, 1932, **35**, 973.

Beef heart, obtained the day of slaughtering, is freed of fat; the muscle and fibrous tissue are finely minced, and weighed. To each pound of mince 1 liter of tap water is added. The mixture, in an enamel container, is infused in the ice-box for 16 hours, then placed over a low flame and brought slowly to 85°C., where it is maintained for 30 minutes. The infusion is filtered through Prat Dumas filter paper and the meaty pulp squeezed very dry. The resulting infusion should equal the original volume of water. Twenty gm. of Difco proteose peptone is dissolved in each liter of infusion; the reaction is adjusted to pH 8 (about 30 cc. of N/1 NaOH per liter). It is filtered through No. 12 Whatman filter paper into flasks, and sterilized in an Arnold apparatus on 3 successive days, allowing 30 minutes for each liter of broth or less in the container.

Multiples of the following salt-dextrose solution are prepared:

Dextrose	2 gm.	All chemicals of Reagent Purity.
$NaHCO_3$	2 "	
NaCl	2 "	
$Na_2HPO_4{:}12\,H_2O$	1 "	
Distilled water	50 cc.	

This solution is filtered through a Berkefeld candle, tested for sterility, and then aseptically pipetted into the broth in the proportion of 50 cc. to 1 liter.

As a precautionary measure the completed broth should be incubated 2 days before inoculating to insure sterility.

At 9 a. m. a tube of 0.1 or 0.2% dextrose broth is inoculated with a suitable hemolytic streptococcus, placed in the water bath and shaken hourly, thus insuring a rapid growth. At 4 p. m. 6 cc. of this vigorously growing culture is added to 1 liter of the broth, previously warmed to 37°. This is well shaken and incubated overnight. The next morning it is filtered through an "F" Chamberland filter, by placing the candle in a long tube containing the culture and drawing the broth from the outside to the inside, and thence into a sterile flask which is connected to the house vacuum. The first 100 cc. is discarded because it contains little or no streptolysin.

As shown by Neill and Mallory[5] streptolysin is active only in the reduced state. Todd's method for reducing it is as follows: To each liter of filtrate add 10 cc. of N/1 NaOH and 1 gm. of sodium hydrosulphite which has been freshly ground to a fine white powder; this is dropped on to the surface of the filtrate and dissolves slowly. The flask is stoppered and connected to a high vacuum pump; a high vacuum is maintained for 2 or 3 hours until only very few bubbles are given off the fluid. The reduced hemolysin is pipetted into 50 or 100 cc. flasks, covered with about 2 inches of sterile melted vaseline and stored in the ice-box. Todd recommends storing the unreduced streptolysin in the cold, and reducing the required amount the day it is to be used; but we have found that the hemolytic titer is usually higher several days after reduction than immediately after being reduced; and also that aged reduced streptolysin is more stable than that tested on the day of reduction.

The streptolysin is tested for hemolytic power in the following amounts: 0.4, 0.35, 0.3, 0.25, 0.2, 0.16, 0.14, 0.12, 0.1, 0.08, 0.06, 0.04. Then saline is added to make a total volume of 1.5 cc. Each tube is well shaken—then 0.5 cc. of 5% washed red blood cells (rabbit) is added. To obtain a very accurate end point, *i. e.,* complete hemolysis, each tube is shaken individually for about 30 seconds *immediately* after adding the red cells. An end point at about 0.12 to 0.08 is usually obtained by this method.

The streptolysin thus produced is a satisfactory reagent for testing antibodies in serum. We have not tested its antigenic capacity; but as Todd[3] has shown that serum-free streptolysin is antigenic it seems probable that this lysin would induce antibodies when injected into animals. Only certain strains of hemolytic streptococci produce suitable streptolysin.

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[5] Neill, J. M., and Mallory, T. B., *J. Exp. Med.,* 1926, **44**, 241.

Summary. A very satisfactory medium for producing streptolysin and yielding heavy growths of bacteria results from adding filtered sterile salts, buffers, and glucose to beef heart, peptone broth previously sterilized at 100°C.

6778

Failure of a Mouse Carcinoma Material to Enhance a Mouse Sarcoma.

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From the Laboratories of the Rockefeller Institute for Medical Research.

It has been shown that an homologous rabbit carcinoma enhancing material[1, 2] failed to enhance a carcinoma of the mouse.[3] Haaland,[4] Leitch,[5] and Casey[3] have demonstrated, however, that a material can be obtained from the same mouse carcinoma (No. 63) which will enhance both the incidence and the growth of the primary tumor when used in conjunction with the tumor inoculation. It seemed desirable, therefore, to determine whether homologous enhancing materials will enhance heterologous transplanted tumors within the same animal species. Since other transplantable rabbit tumors are not available, the experiments were carried out with mouse tumors. In the 2 experiments here reported the enhancing material was obtained from the Bashford mouse carcinoma (No. 63) and its effect was observed in connection with implants of mouse sarcoma (No. 180). Two different batches of preserved (enhancing) material and sarcoma emulsion were used; 60 mice of the Rockefeller Institute strain were employed; in the first experiment, there were 40 females and in the second 20 males. Thirty of these mice were injected with a saline emulsion of Bashford mouse carcinoma (No. 63) tissue which had been preserved anaerobically at 24°F. for 42 days.[3] Two weeks later the entire 60

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[1] Casey, A. E., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 816.

[2] Casey, A. E., *J. Exp. Med.*, 1933, in publication.

[3] Casey, A. E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 674.

[4] Haaland, M., *Lancet*, 1910, **1**, 787.

[5] Leitch, A., *Lancet*, 1910, **1**, 991.

mice were inoculated into the left groin with 0.09 cc. of an emulsion (10 parts saline, 1 part tumor) of fresh mouse sarcoma (No. 180). Measurements of the local growths were made at 6, 12, and 16 days. The ulceration of many tumors after this time precluded further measurements.

For the sake of clarity the mice treated with the preserved carcinoma material and later inoculated with the sarcoma are called the "experimental" mice, and the mice inoculated only with the sarcoma the "control" mice. At 16 days, 16 of 28 experimental mice (57%) had primary tumors which averaged 0.343 cc. in volume. This compared with 22 primary tumors (73%) among the 30 control mice averaging 0.509 cc. The size of the primary tumors per animal inoculated was 0.196 cc. among the experimental mice as compared with 0.373 cc. among the controls. The results, therefore, show no evidence that the sarcoma was enhanced (there is an insignificant suggestion of an inhibition) and are in marked contrast to the previous results in which the preserved mouse carcinoma material showed distinct evidence of enhancement of the same Bashford carcinoma (No. 63) in 10 consecutive experiments.[3] Further experiments designed to detect and differentiate enhancing materials with a view to their specificity should be attempted.

6779

Growth Characteristics of Rough and Smooth Acid Fast Bacteria Living in Microculture.

RALPH W. G. WYCKOFF. (Introduced by Peyton Rous.)

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While studying the growth of a number of acid fast bacteria in microculture a series of micro motion pictures has been made of a strain of organisms pathogenic to frogs. This mycobacterium, *M. ranae 1*, was provided by the Phipps Institute which obtained it several years ago from E. R. Baldwin. Planted at room temperature on the usual media it gives a rich white growth which at first often has the consistency of very thick cream but with age assumes the wrinkled growth more familiar in acid fast bacterial cultures. Both young and old growths are completely acid fast when stained by the Cooper modification[1] of the Ziehl-Neelson technique.

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[1] Cooper, F. B., *Arch. Path. Lab. Med.*, 1926, **2**, 382.

Dissociation was readily brought about by following Petroff's procedure[2] of dilution platings on egg medium containing sodium taurocholate. The rough strain gives the usual wrinkled surface growth on fluid media. Streaked on solid it yields a coarse mealy mass. The smooth strain clouds glycerinated broth and produces a luxuriant mucoid growth on solid media. By transplantation from old Petroff bottles 2 S strains have recently been isolated which differ greatly from one another in their mucosity, one being so fluid that it will almost drip from the surface of an inverted Petri dish.

Micro motion picture records show that these R and S dissociants grow in very different ways. The completely dissociated R organisms lie closely packed together as they multiply. They show little tendency to spread over the medium and commence early to overlie one another, forming thereby a thick tangled mass of bacilli. Micro colonies of the S organisms thus far photographed, on the other hand, strikingly resemble those of highly capsulated pneumococci. Several bacilli placed close together when seeding a micro field will not elongate for the first day or two. Instead, after a brief lag period they begin to move apart, apparently owing to the elaboration of a large volume of highly viscous material. Subsequent elongation and division result in chains of bacteria separate from adjacent chains by several bacterial diameters. When displaced by the crowding that results from continued multiplication, the chains move without touching one another. At this time they often are seen to oscillate in exactly the fashion to be expected of solid particles suspended in a slowly flowing jelly. The refractive index of this substance bathing the bacteria appears to rise as the culture ages because, while the seed organisms are at the outset black and highly refractile, their progeny rapidly become more and more transparent.

These observations together with other phenomena clearly shown in the motion pictures make it evident that the smooth strains of *M. ranae I* produce a voluminous substance which has no counterpart in the growth products of the rough variant. Such a difference between R and S acid fast organisms is not limited to the frog bacillus. Dissociated strains of the turtle bacillus, *M. chelonei,* behave similarly though the S strain thus far obtained is not so fluid, and its individual organisms are separated by distances not so great as those found with *M. ranae I*. Furthermore, preliminary photographs of a smooth culture of avian tubercle bacilli made by K. C. Smithburn and the writer indicate that a similar material is elaborated by this organism. In view of the established[3] greater pathogenicity of the S than of the R strains of *B. tuberculosis avium*, certain possible implications of these observations are obvious. They are being examined and will be discussed in a later more detailed description of this work.

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[2] Petroff, S. A., and Steenken, W., *J. Exp. Med.*, 1930, **51**, 831.

6780

Total and Thyroxine Iodine Content of Thyroid Gland After Injection of Saline Anterior Pituitary Extracts.

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From the Department of Biological Chemistry and the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

The striking hyperplasia of the thyroid gland produced experimentally by injection of anterior pituitary extracts[1] affords an unusual opportunity to study the intermediate iodine metabolism of the hyperplastic thyroid gland under adequately controlled conditions. It is now well established that following parenteral administration of anterior pituitary extracts there is a decrease in the total iodine content of the thyroid gland, as shown in the dog,[2, 3] guinea pig,[4] duck,[5] and chick.[6] Closs, Loeb and MacKay[4] investigated the nature of this decrease in total iodine further by alcoholic fractionation of pooled thyroid glands of guinea pigs previously treated with anterior pituitary extract. They found a consistent and significant decrease in the alcohol-insoluble or "organic" fraction of the total thyroid iodine. Grab[7] employed the Reid Hunt acetonitrile test to compare the protective potency of thyroid glands of dogs treated with anterior pituitary preparations with that of untreated dog thyroids. The results were variable, but as the average potency of thyroid glands of injected animals was lower, Grab also concluded that the decrease in iodine content occurs chiefly at the expense of the physiologically active or organic iodine fraction. Paal and Huber,[8] however, who used much the same procedure on rats found an increase in the potency of glands after treatment.

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[3] Winn, W. A., and Petroff, S. A., *J. Exp. Med.*, 1933, **57**, 239.

[1] Literature reviewed by Loeb, L., *Klin. Woch.*, 1932, **11**, 2121.

[2] Loeser, A., *Klin. Woch.*, 1932, **10**, 2047; *Arch. exp. Path. u. Pharmakol.*, 1931, **163**, 530.

[3] Grab, W., *Arch. exp. Path. u. Pharmakol.*, 1932, **167**, 413.

[4] Closs, K., Loeb, L., and MacKay, E., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 170; *J. Biol. Chem.*, 1932, **96**, 585.

[5] Schockaert, J. A., and Foster, G. L., *J. Biol. Chem.*, 1932, **95**, 89.

[6] Unpublished data.

[7] Grab, W., *Arch. exp. Path. u. Pharmakol.*, 1932, **167**, 313.

By utilizing the Leland and Foster method for the determination of thyroxine,[9] a more satisfactory partition of the iodine compounds known to be present in alkaline hydrolysates of thyroid gland can now be made. It was hoped that by using sufficiently large animals (sheep), it would be possible to estimate the total iodine, inorganic iodine and thyroxine content of a single lobe, thus permitting the removal of one lobe before treatment as a control for the second lobe, removed after treatment. The changes in the second lobe could then be compared with similar data available for normal human[9, 10] and hog[6] thyroid glands and for diffuse and nodular toxic and non-toxic goiters.[10]

Procedure. Five adult female sheep weighing about 150 pounds were obtained and one lobe of the thyroid gland removed, without preliminary iodine feeding and without using iodine at operation. The operated animals were kept in a separate enclosure on our experimental farm (May-June, 1932). They were permitted to graze freely, oats and water, but no salt or mineral feed, being added to the diet. Beginning 5 days after hemithyroidectomy, 4 sheep were injected intraperitoneally with an average of 50 cc. of a saline anterior pituitary extract (equivalent to 17 gm. of fresh beef anterior pituitary lobes) every day for 10 days. The day after the last injection, the second lobe of the thyroid gland was removed.

The thyroid glands thus obtained were weighed, a small piece removed for sectioning, the remainder minced, dried at 60°-65°C. to constant weight and pulverized. Duplicate samples of 10 mg. were weighed out on a micro-balance for determination of *total iodine* by a micro-method previously described by one of us.[5] The remainder of the lobe was weighed, the desiccated gland thoroughly extracted with iodine-free water,[11, 10] and the *iodine in the aqueous extract* determined. The water-insoluble residue was analyzed for *thyroxine iodine* by the method of Leland and Foster.[9]

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[8] Paal, H., and Huber, W., *Arch. exp. Path. u. Pharmakol.*, 1931, **162**, 521.

[9] Leland, J. P., and Foster, G. L., *J. Biol. Chem.*, 1932, **95**, 165.

[10] Gutman, A. B., Benedict, E. M., Baxter, B., and Palmer, W. W., *J. Biol. Chem.*, 1932, **97**, 303; Trans. Am. Assn. Study Goiter, 1932, 31.

[11] Harington, C. R., and Randall, S. S., *Quart. J. Pharm. and Pharmacol.*, 1929, **2**, 501.

Anterior pituitary extract was prepared by soaking the carefully trimmed anterior pituitary lobes for 5 minutes in 95% alcohol, rinsing with sterile saline solution, mixing the thoroughly ground anterior lobes with twice their weight of sterile physiological salt solution and centrifuging. The pink, turbid supernatant fluid was kept on ice until ready for use. The potency of the extracts used was proven by tests on chickens about 8 weeks old and averaging 400 gm. in weight (Table I).

TABLE I.
Effect of Saline Anterior Pituitary Extracts on Thyroid Gland of Chicken.

	Chicken No.	Dry weight one lobe mg.	Total iodine one lobe γ	Total iodine % dry gland
First extract				
Controls	1	14.8	115	.776
	2	12.4	85	.685
2 cc. injected	3	31.0	7	.023
daily 5 days	4	27.3	4	.015
Second extract				
Controls	5	5.6	24	.430
	6	7.5	22	.300
3 cc. injected	7	23.9	1	.004
daily 6 days	8	21.2	1.5	.007

Results. On histological examination, characteristic hyperplastic changes, including hypertrophy of the Golgi apparatus, were observed in every thyroid gland after anterior pituitary injections. They were more marked in the glands showing more pronounced chemical changes. We are indebted to Dr. A. Severinghaus for the special stains.

The chemical results (Table II) indicate a consistent decrease in total iodine per cent of dry gland after injection of anterior pituitary extract, under conditions which led to an increase in iodine in our untreated control sheep. The mean decrease of 50% is much less than that found in ducks and chicks but comparable to that reported in dogs (75%)[3] and guinea pigs (65%)[4]. There was a proportionate and consistent decrease in thyroxine iodine per cent of dry gland following anterior pituitary injection. The reduction in the absolute content of total iodine and thyroxine iodine per lobe after injection is less marked than the relative decrease, due to the compensatory enlargement of the gland. The changes in water-extractable iodine (an approximation of the inorganic iodine content) are not regarded as significant. The figures for glands 5a and 8a are undoubtedly too high. Subsequent experience has shown that the

TABLE II.
Effect of Saline Anterior Pituitary Extract on Thyroid Gland of Sheep.

Sheep No.	Dry weight, gm.	Dry matter, %	Total iodine % dry gl.	Thyroxine Iodine % dry gl.	Iodine in aqueous extract, % dry gl.	Thyroxine iodine % of org. I	Iodine in aqueous extract, % of tot. I	Total iodine per lobe, mg.	Thyroxine iodine per lobe, mg.
4 a	.498	24.9	.31	.065	.013	21.9	4.2	1.54	.324
b	.576	15.4	.07	.017	.006	26.6	8.6	0.40	.098
5 a	.675	24.9	.27	.082	.056?	38.3		1.82	.554
b	.677	20.0	.19	.044	.009	24.3	4.7	1.29	.298
6 a	.454	25.0	.28	.078	.025	30.6	8.9	1.27	.354
b	.672	17.4	.19	.040	.013	22.6	6.8	1.28	.269
7 a	.353	25.8	.44	.111	.015	26.1	3.4	1.55	.392
b	.542	17.0	.16	.030	.013	20.4	8.1	0.87	.163
Control sheep (not injected)									
8 a	.366	24.1	.12	.023	.052?	33.8		0.44	.084
b	.556	24.8	.23	.055	.020	26.2	8.7	1.28	.306

a = control lobe removed before anterior pituitary injection.
b = lobe removed after anterior pituitary injection.

aqueous extract of desiccated thyroid contains small amounts of protein iodine and occasionally, as in these 2 instances, large amounts of organic iodine may be extracted with water.

The data indicate a depletion of the thyroglobulin content of the thyroid glands after 10 daily injections of anterior pituitary extract, (Fig. 1) presumably due to increased liberation of thyroid hormone into the circulating fluid. This conclusion is in agreement with earlier studies on the total iodine content of the thyroid gland and its fractionation with alcohol.[1-5] It is also in harmony with interpretations of histological changes in the thyroid gland,[1, 5] and with the loss of weight, increase in metabolism,[12, 13] increased alcohol-insoluble iodine in the blood[4, 14] and other effects of anterior pituitary injections.

It has been suggested that, in view of the striking analogies between the effects of anterior pituitary injections and changes observed in Graves' disease, the pituitary gland and associated structures in the mid-brain are primarily affected in Graves' disease. The only observation made by us relevant to this problem is that we

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[12] Siebert, W. J., and Smith, R. S., *Am. J. Phys.*, 1930, **95**, 396.

[13] Verzár, F., and Wahl, V., *Biochem. Z.*, 1931, **240**, 37.

[14] Schneider, E., *Deutsche Z. f. Chir.*, 1932, **236**, 391.

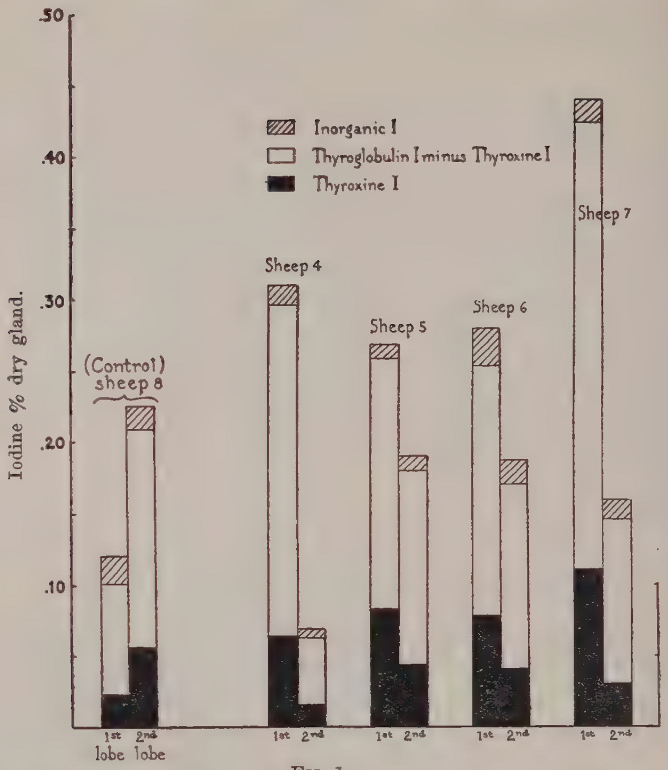

FIG. 1.

The effect of saline anterior pituitary extract on iodine fractions of the thyroid gland of sheep. The water-extractable iodine of 8a and 5a has been represented as equal to the percentages of water-extractable iodine in 8b and 5b.

found no significant decrease in thyroxine iodine per cent of organic iodine such as has been observed in many cases of severe Graves' disease.[15, 10] Since we succeeded in producing a decrease in total iodine comparable to the low levels found in Graves' disease in only one instance, this negative result does not preclude the possibility that such a change in thyroglobulin may occur.

Summary. After 10 daily intraperitoneal injections of a saline anterior pituitary extract, there is a decrease in the total iodine content of the thyroid gland of the sheep, with a proportionate decrease in its thyroxine content.

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[15] Wilson, L. B., and Kendall, E. C., *Am. J. Med. Sci.*, 1916, **151**, 79.

6781

Influence of Salt Upon Diffusion from Bacterial Cells.

C.-E. A. WINSLOW and H. H. WALKER.

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Numerous experiments in this laboratory and elsewhere[1] have shown that all cations in low concentration stimulate, and in higher concentration inhibit, bacterial viability. It has been suggested that the stimulating effect was associated with increased diffusion through the cell wall—the inhibitive effect with decreased diffusion. Shaughnessy and Winslow[2] demonstrated that salt concentrations known to be favorable did show an increase in ammonia in the menstruum surrounding the cells while toxic concentrations showed a reverse effect. No bacterial counts were made in these experiments, however; and the purpose of the present study was to check these earlier results by parallel determinations of bacterial numbers and diffusion products.

Heavy cultures of *Esch. coli* were washed off in distilled water, from 12-18 hour agar surface growths in Blake bottles, filtered through paper or cotton to remove clumps or agar, and centrifuged twice to remove soluble foreign materials. The washed cells were then divided into 4 portions which were resuspended respectively in distilled water, in 0.05-0.08 Molar NaCl, in 0.8-1.0 Molar NaCl and in 2.0 Molar NaCl. They were allowed to stand in contact with these menstrua for 30 minutes at 37°. The cells were then removed by centrifugation and the ammonia in the menstruum determined by direct Nesslerization after preliminary clarification by the Standard Methods for Water Analysis of the A.P.H.A. with slight modification. This modification consisted of comparing each specimen with a separate set of color standards containing a corresponding amount of clarified NaCl, since it was found that the different concentrations of NaCl employed caused different degrees of interference with normal Nessler color development.

The basic data for 5 experiments are presented in Table I, with computations of rate of ammonia yield per cell per hour based on the mean of the initial and final counts.

The initial counts of course varied in different experiments; and

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[1] Reviewed by Winslow and Dolloff, *J. Bact.*, Balt., **15**, 67; Winslow and Haywood, *J. Bact.*, Balt., **22**, 49.

[2] Shaughnessy and Winslow, *J. Bact.*, Balt., **14**, 69.

TABLE I.

Exp.		1	2	3	4	5
Initial bacteria, billions per 10 cc.		151	257	48	97	187
Final bacteria, billions per 10 cc.	0.0 NaCl	118	284	41	89	176
	0.05-0.08 M Nacl	157	316	37	92	198
	0.8-1.0 M NaCl	114	220	25	74	62
	2.0 M NaCl	67	198	26	76	127
Ammonia nitrogen, mg. per 10 cc.	0.0 NaCl	.013	.016	.008	.016	.027
	0.05-0.08 M NaCl	.020	.022	.011	.017	.038
	0.8-1.0 M NaCl	.011	.013	.005	.008	.021
	2.0 M NaCl	.007	.009	.003	.009	.018
Ammonia nitrogen, mg. x 10^{-11} per cell per hour	0.0 NaCl	.019	.012	.037	.034	.030
	0.05-0.08 M NaCl	.026	.015	.052	.035	.039
	0.8-1.0 M NaCl	.017	.011	.027	.018	.034
	2.0 M NaCl	.013	.008	.017	.021	.023

the amount of diffused ammonia per cell per hour also varied somewhat widely from experiment to experiment in a given menstruum. Such variation might be expected on account of differences in initial vitality of culture, degree of clumping, etc.; but the relative results for different menstrua in a given experiment check closely. In every instance but one the dilute NaCl solution (0.05-0.08 M) showed a higher final count than the distilled water and in every instance it showed greater ammonia diffusion per cell. In every instance the medium strength salt (0.8-1.0 M) showed lower final counts than the distilled water and in every instance but one it also showed a lower rate of ammonia diffusion per cell. The strongest salt (2.0 M) showed sometimes higher and sometimes lower counts than the 0.8-1.0 M concentration (which might be expected from the sharp variations in numbers which occur in such salt concentration[3]) and ammonia diffusion was in all but one instance less. These relative relationships remain essentially the same when, as a check procedure, ammonia production per cell per hour is computed on the basis of either initial or final counts rather than on the mean between these values.

The percentage results presented in Table II make these relations clearer.

It seems evident that the influence of salt content upon bacterial viability runs closely parallel to its effect on diffusion of ammonia

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[3] *J. Bact.*, Balt., **24**, 185.

TABLE II.
Influence of Salt upon Bacterial Viability and Diffusion of Ammonia into the Menstruum in Terms of mg. per Cell per Hour Expressed as Percentages of Results in Salt-free Control.

	M NaCl	Exp.					Average
		1	2	3	4	5	
Final Bacterial Count	0.05-0.08	133	111	90	103	112	110
	0.8-1.0	97	77	61	83	35	71
	2.0	57	70	63	85	72	69
Diffused Ammonia per Cell per Hour	0.05-0.08	137	125	141	103	130	127
	0.8-1.0	89	92	73	53	113	84
	2.0	68	67	46	62	77	64

outward through the cell wall. The short time period employed, in the absence of nutrient materials, makes it reasonably clear that changes in production of ammonia within the cell are not the determining factors. We may conclude then, that a dilute stimulating concentration of NaCl increases and a strong toxic concentration decreases diffusion of metabolic products outward through the cell wall. Whether this is due to an influence on the permeability of the cell membrane or to an effect on physico-chemical conditions within or without the cell is uncertain.

6782

Cellular Responses to Acetone-Soluble Lipoids from Mycobacteria.

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The cellular responses to phosphatides and the so-called waxes isolated by Anderson[1] from acid-fast bacilli have been studied by Sabin and Doan,[2] Sabin, Doan and Forkner,[3] and by Smithburn and Sabin.[4] Anderson[1] has separated from the bacilli a third lipoidal component which is soluble in acetone and which is recovered as a soft brown, salve-like solid with an odor not unlike that which arises from cultures of tubercle bacilli. This substance was shown[1] to consist largely of a mixture of fatty acids. The cellular responses induced by this acetone-soluble lipoid will be discussed in the present communication.

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[1] Anderson, R. J., *Physiol. Rev.*, 1932, **12**, 166.

[2] Sabin, F. R., and Doan, C. A., *J. Exp. Med.*, 1927, **46**, 645.

[3] Sabin, F. R., Doan, C. A., and Forkner, C. E., *J. Exp. Med.*, 1930, **52**, Suppl. No. 3, 3.

[4] Smithburn, K. C., and Sabin, F. R., *J. Exp. Med.*, 1932, **56**, 867.

Rabbits were used in the experiments. The lipoids employed were isolated from human, bovine, and avian tubercle bacilli, and the timothy grass bacillus and were injected intraperitoneally. The acetone-soluble lipoid is not miscible with water and was therefore suspended in mineral oil for administration in the first experiments. Later it was made neutral to litmus with N/10 NaOH and suspended in water for injection. The material was also given undiluted. Cellular studies were made of the fresh tissues by the supravital and by the fixed tissue methods.

The acetone-soluble fat was found to be the most irritating of the crude lipoids from acid-fast bacteria. It induced extreme vascular dilatation, proliferation, and hemorrhages. It is the only substance from tubercle bacilli yet tested in this laboratory which has produced massive adhesions. Perhaps due to the content of phthioic acid,[1, 3] it induced the formation of epithelioid cells in considerable numbers. In addition, this lipoid caused marked stimulation and proliferation of every type of connective tissue cell and it always called forth leucocytes in large numbers. Many of the leucocytes were contained in clasmatocytes; others were free. Red blood cells and droplets of mineral oil were also seen within clasmatocytes. Many undifferentiated connective tissue cells, fibroblasts, lymphocytes, and plasma cells also characterized the reactions. No particular type of cell was dominant in the reaction. These changes were noted after administration of the lipoid undiluted and also when it was suspended in mineral oil.

In subsequent experiments the acetone-soluble fat was made neutral to litmus with N 10 NaOH and suspended in water for injection. This procedure brought about a marked reduction in the irritating properties of the lipoid. After administration of the neutralized material, only a minimal number of epithelioid cells appeared in the tissues. There was also a diminution in the proliferation of other types of cells, as well as in the vascular changes. However, neutralization did not reduce the power of the lipoid to induce the formation of adhesions which have been found to persist for at least one month. In this connection it is interesting to note that, whereas the phosphatide and fatty acid I (phthioic acid) from tubercle bacilli produced epithelioid cells in great numbers,[3] this property was lost when the substances were neutralized with KOH.[5]

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[5] Sabin, F. R., and Smithburn, K. C., unpublished data.

Unquestionably some of the irritating properties of the tuberculolipoids are largely due to the presence of free fatty acids.

6783

Tetanus Toxoid in Prophylaxis against Tetanus.

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Ramon[1, 2, 3] and his colleagues have applied their method of detoxifying diphtheria toxin with formaldehyde to the detoxification of tetanus toxin. The addition of 0.3 to 0.4% of commercial formaldehyde to tetanus toxin causes detoxification at incubator temperature in about 2 weeks. A tetanus toxin containing 5,000 to 10,000 MLD per cc., for guinea pigs, when detoxified can be injected subcutaneously into guinea pigs weighing 300 to 350 gm., in doses of 5 cc. without giving rise to symptoms of intoxication.

Ramon and his colleagues state that 3 doses of 1 cc. of toxoid injected subcutaneously into guinea pigs at 14 day intervals induce a high degree of immunity.

The purpose of this study was to ascertain the protective power and immunity afforded to guinea pigs by 3 doses of tetanus toxoid. The degree of protection obtained was determined by measuring the neutralizing power of the serum of the immunized guinea pigs when administered to normal guinea pigs combined with 0.1 L+ dose of standard tetanus toxin.

We studied the immunizing effects of 2 toxoids, one No. 6 prepared from a toxin containing 3,000 MLD per cc., and the other, prepared from a toxin containing 10,000 MLD per cc. Titration of the sera of 3 guinea pigs immunized with toxoid No. 6 indicated the presence of 0.2 of a unit of antitoxin per cc. of blood-serum, while titration of the sera of 5 guinea pigs immunized with toxoid No. 7 indicated the presence of 0.5 of a unit of antitoxin per cc. of blood serum.

On the basis of the titrations of the antitoxic content of the sera of the guinea pigs treated with toxoids No. 6 and No. 7, the animals received the following doses of tetanus toxin:

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[1] Ramon, G., *Ann. d., Inst. Pasteur.*, 1924, **38**; *C. R. Acad. Sci.*, 1924, **78**, 436.

[2] Descombey, F., *C. R. Soc. Biol.*, 1924, **91**, 239.

[3] Zoeller, Chr., and Ramon, G., *Presse Med.*, 1926, **164**, 485.

TABLE I. Toxoid No. 6.

No.	Wt. approx. gm. × 100	MLD of Toxin × 1000	Results
1	5	6	Survived
2	5	10	”
3	5	15	”

TABLE II. Toxoid No. 7.

No.	Wt. approx. gm. × 100	MLD of Toxin × 1000	Results
1	5	9	Survived
2	5	15	”
3	5	15	”
4	5	20	D. Tetanus
5	5	20	” ”

The experiments which form the basis of this report confirm the claims of Ramon and his associates that tetanus toxin can be detoxified with commercial formaldehyde as shown by the absence of symptoms from injections into guinea pigs of 5 cc. amounts of a toxoid prepared from toxins containing 3,000 to 10,000 MLD per cc.; that 3 doses of 1 cc. of such toxoid when injected subcutaneously into guinea pigs at 14 day intervals stimulate the production of from 0.2 to 0.5 unit of antitoxin per cc. of serum; and that the antitoxin content of the blood of the animals protects them against 10,000 to 15,000 MLD 2 to 6 months subsequent to the administration of the toxoid.

Ramon and Zoeller[4] tested the antitoxin titer of the sera of 12 individuals, 4 to 5 years subsequent to the injection of 2 or 3 doses of tetanus toxoid, and found the titer to range from 1/500 to 1/6 of an American unit (1/250 to 1/3 international unit). In another individual who received a fourth dose, sometime after the usual course of immunization, the antitoxin titer was 1.5 units per cc. of serum.

In the titration of the antitoxin content of the serum of the immunized guinea pigs an average of 1/5 unit per cc. of serum was found in the animals immunized with the weaker toxoid, and 1/2 unit per cc. of serum in the animals immunized with the stronger toxoid. One cc. of the serum of each group of guinea pigs neutralized as much as 750 MLD of tetanus toxin.

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[4] Ramon, G., and Zoeller, Chr., *C. R. Soc. Biol.*, 1933, **112**, 347.

6784

Hypertension with Arteriolar and Glomerular Changes in the Albino Rat Following Subtotal Nephrectomy.

J. EDWIN WOOD, JR., AND CLAYTON ETHRIDGE. (Introduced by J. R. Cash.)

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Chanutin and Ferris[1] have already reported hypertension and renal insufficiency in rats following partial nephrectomy. This report describes briefly the histological changes in the kidney stump of rats at intervals after subtotal nephrectomy and records additional blood pressure observations.

In this experiment 184 Wistar strain albino rats were studied. In 157 of these, approximately two-thirds of the left kidney was removed by polar ligation, and the remaining right kidney was excised one week later. Of 27 control animals, each had 2 laparotomies (one week apart) when the kidneys were exposed, handled and replaced intact. After the operative procedures all of the animals were fed on a normal balanced diet containing approximately 20% protein. At intervals varying from one to 281 days after second operation, a direct carotid blood pressure reading was made (ether anesthesia), and autopsy then performed. Infection was not encountered except in rare instances, and did not affect the results reported below. Tissues were fixed variously in formol, mercuric chloride, and Helly's fluid. Frozen and paraffin sections were stained with Scharlach-R, Mallory-Heidenhain, and other usual methods.

The blood pressure in the 27 control rats ranged from 100 to 140 mm. Hg., with an average of 120 mm. Hg. (a figure already fully established by Chanutin and Ferris). Of 157 animals with subtotal nephrectomy, 10 had blood pressures ranging from 70 to 99 mm. Hg.; 57, from 100 to 139 mm. Hg.; 45, from 140 to 169 mm. Hg.; 34, from 170 to 199 mm. Hg.; and 11, from 200 to 230 mm. Hg. In general, the test animals with subnormal blood pressures were acutely ill. The higher blood pressure levels were found in rats with subtotal nephrectomy of 60 days or longer. Of 34 test animals killed from one to 59 days after second operation, only 5 showed blood pressures of 140 mm. Hg. or above, whereas, of 123 animals killed from 60 to 275 days after second operation, 85 (or 69%)

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[1] Chanutin, A., and Ferris, E. B., *Arch. Int. Med.*, 1932, **49**, 767.

showed blood pressures of 140 mm. Hg. or above. In each instance of hypertension, degenerative glomerular changes have been present.

Microscopic studies show constant and characteristic changes in the renal stump. During the first 12 days after second operation there is rapid dilatation of the tubules, with marked fatty change and hyperplasia of their epithelium. The glomeruli show no appreciable change, other than increase in size, until about 40 to 70 days after second operation. About this time there is a thickening of the supporting structures of the glomeruli, as well as swelling and fatty change of the glomerular epithelium with formation of capsular adhesions (Fig. 1). One has the general impression that the media of many of the smaller arteries and arterioles is definitely thickened. More striking, however, are focal accumulations of fat within the media of smaller arteries and arterioles, frequently leading to marked diminution in the size of the lumen. The arterial lesions (Figure 2) bear a striking resemblance to those of human renal arteriolosclerosis. Progression of all of these changes leads to complete destruction of many glomeruli with atrophy of their related tubules. The number of completely destroyed renal units increases with the duration of the experiment. The remaining intact

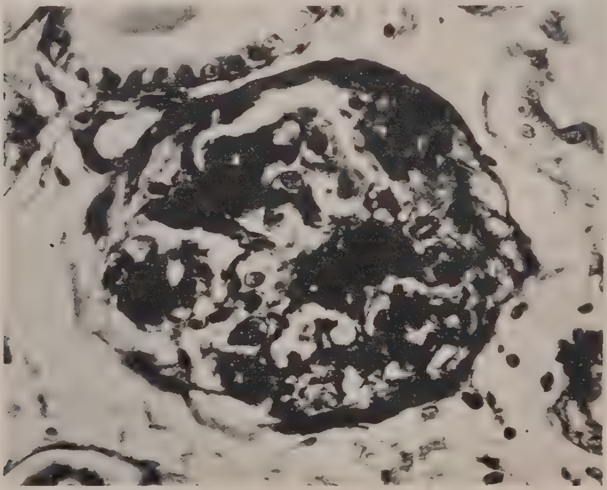

FIG. 1.

Rat 360 B.E. Mallory-Heidenhain Azan Carmine Stain. Showing a moderately advanced glomerular lesion with degeneration, fibrosis and hyalinization. ×470.

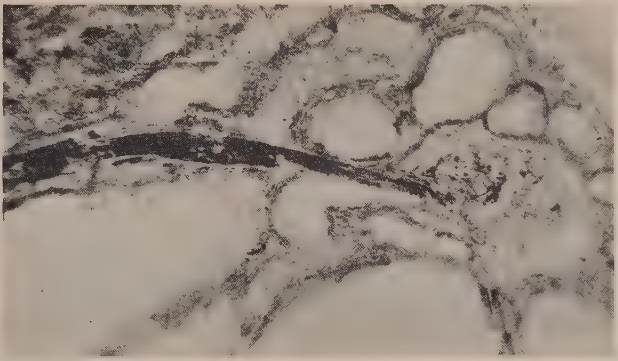

FIG. 2.
Rat 352 B.N. Scharlach-R and Haematoxylin Stain. Fat which photographs black is seen in afferent arteriole and glomerulus. ×95.

renal units show tremendous hypertrophy of both glomeruli and tubules.

None of these pathological changes has been seen in the kidneys of 27 control animals or in the kidneys of 13 animals subjected to unilateral nephrectomy. The resemblance of the hyaline glomerular changes to amyloid formation in some instances caused us to test for this substance with iodine-sulphuric acid, methyl violet, and intravenous injection of congo red.[2] Negative results have been obtained by all methods.

From the preceding data it appears fair to conclude that chronic progressive glomerular and arterial renal lesions, associated with hypertension, have been produced in rats by subtotal nephrectomy. In our opinion these changes may be interpreted as a natural sequence of hypertrophy and degeneration brought about by functional strain.

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[2] Smetana, H., *Johns Hopkins Hosp. Bull.*, 1925, **37**, 383.

6785

Relationship of Acidogenic Bacteria to Diet in Production of Dental Caries in the White Rat.*

J. L. ETCHELLS AND E. D. DEVEREUX. (Introduced by W. L. Mallmann.)

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It has been noted by Hoppert *et al*[1] that white rats which received comparatively coarse foods were more prone to develop dental caries than those animals receiving a finely ground ration. This was explained on the basis of impaction: the coarse food particles became firmly lodged in or between the teeth and caries developed following the fermentation of these particles. The finely ground food was not subject to impactions; as a result dental caries did not develop in the absence of a fermentable material.

In this experiment an attempt was made to determine the ability of certain acid producing organisms in pure cultures to produce caries when introduced in connection with a sterilized coarse ration. The following cultures were employed:

No. 1. *Lactobacillus acidophilus*, oral strain, isolated from an old rat with extensive caries.

No. 2. A rough type of *L. acidophilus*, from the mouth of a human having dental lesions.

No. 3. A cocco-bacillus from same source as No. 2.

No. 4. A coccus of fowl origin, isolated in pure culture from a fowl having a partially eroded beak.

The diet chosen for the rats was one that was adequate for growth, retained its physical characteristics upon sterilization and was palatable. The formula for the ration in percentages by weight was as follows: Alfalfa 10, corn 63, oilmeal 15, yeast 5, casein 5, sodium chloride 1, and calcium carbonate 1.

The ration after having been thoroughly mixed, was placed in suitable containers, plugged with cotton and autoclaved for 30 minutes at 15 pounds pressure. A control container was plated for bacterial growth to insure sterility. After being sterilized the food was broken up with a sterile glass rod. One cc. quantities of 24-hour yeast extract broth[2] cultures of the organisms were added to the rations daily. The control animals received both sterile and non-sterile coarse rations and a finely ground non-sterile ration.

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* Paper No. 158 (n. s.), from the Michigan Agricultural Experiment Station

[1] Hoppert, C. A., *et al.*, *J. Dent. Res.*, 1932, **12**, 161.

[2] Devereux, E. D., *A. J. P. H.*, 1932, **22**, 1293.

After a period of 8 to 10 weeks the animals were etherized, the jaws removed and streak plates made from carious areas of the teeth upon yeast extract agar.[2] Although only 8 animals had the test organisms added to their sterile coarse ration, two for each organism, all displayed extensive dental lesions. Organisms No. 1, 3 and 4 were reisolated from the carious lesions of their respective animals. While the organism isolated from the rats receiving organism No. 2 corresponded closely to that organism culturally and morphologically it was not present in as great numbers as the others when reisolated. It was noted that in the 2 control animals on a sterilized coarse diet dental lesions were negligible in that only one small cavity was found in one of the rats. Two other control rats receiving the unsterile coarse ration, containing the organisms naturally present in the food, displayed extensive dental lesions. In both cases impaction had occurred. However, no caries developed in the 2 control rats which were fed the same unsterile diet in a finely ground condition. This has been explained by Hoppert as being due to the fact that there was no impaction of the food. The results obtained from those animals which received the test organisms are in striking contrast to those obtained from those control animals on a sterilized coarse ration and those on the non-sterile finely ground diet. From this there appears to be a relationship between the mechanical factor of impaction and the relative numbers of significant bacteria present.

It was assumed that in the case of an extensively decayed tooth, in which the cavity proceeded to the bone tissue, that the organisms might be introduced into the blood stream, and give rise to agglutinins. When agglutination tests were conducted in the usual macroscopic manner it was observed that the antigen of organism No. 1 was agglutinated by sera from rats receiving this organism in their diets, the highest titre in each case being 1-256. The sera of the rats which received organisms Nos. 2, 3 and 4 agglutinated their specific organisms only in very low dilutions, 1-1 and 1-2.

In conclusion it might be said that while there are many contributing factors to the causation of dental caries, indications are that in the production of dental caries in the white rat there exists a relationship between the mechanical factors of impaction and the relative numbers of significant bacteria present.

6786

On the Motion of Growth. VII. Fundamental Relations and Quantities of Growth and Metabolism.

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This paper affords, in extension of work already reported,[1] a further description of the fundamental relations and quantities by means of which a dynamical connection between growth and metabolism has been established.

Definition of Growth. Many writers have regarded growth, in the broadest sense, as a matter of increase in size. One of the best expressions of this view has been given by Julian Huxley, who states that growth is "a process of self-multiplication of living substance."[2] For analytical purposes, however, it is better to surrender the position that growth must necessarily or by implication consist substantially only of an increase in size. It is, in fact, useful to approach the problem in more general terms and thus to conceive of growth as involving a *change* rather than exclusively an increase in size. Yet mere change alone cannot itself characterize growth, for this denotes a difference simply, and above all, a difference without respect to the quantity responsible for generating the resulting surplus or deficit as the case may be. Hence, the essence of growth is held to be change in size per unit size; and, since the process has been shown subject to dynamical constraints we have proposed to consider growth a mode of motion defined in practical terms as change in mass per unit mass. Length and cell number may also serve, where suitable or convenient, as valid measures of size and so of growth as well. The significance of the preceding definition is best brought out by comparing, as in Table I, what may, for the purpose in view, be called true growth and ordinary gain.

Minot[3] was among the first to recognize this "relativity of growth" because he laid great stress upon methods designed to approximate the relative rate of gain and clearly perceived that the latter should be called the "rate of growth". But, even though he

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[1] Wetzel, N. C., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 224, 227, 233, 354, 358, 360.

[2] Huxley, J. S., Problems of Relative Growth, New York, 1932, p. 6.

[3] Minot, C. S., *J. Physiol.*, 1891, **12**, 97.

TABLE I.
Comparison between *True Growth* and *Ordinary Gain*.

	Growth	Gain
(1) Definition	Change in Size per Unit Size	Change in Size
(2) Quantity*	$q = \int_0^t \frac{1}{z}\frac{dz}{dt}dt = \text{Log}_e \frac{z}{z_0}$	$\int_0^t \frac{dz}{dt}dt = z - z_0$
(3) Instantaneous Rate	$\frac{dq}{dt} = \frac{1}{z}\frac{dz}{dt}$	$\frac{dz}{dt}$
(4) Unit of Rate	Kg./Kg./T	Kg./T

* Size is represented by z and measured in terms of mass, length or cell number as convenient.

did not define growth, or rather the quantity of growth as here set out, we should ultimately arrive at precisely the statement just given if we proceeded with his method to the limit and integrated the result over some finite interval of time.

The Simplest Law of Growth and Two Amendments. Sufficient experience from various biological fields has accumulated to uphold the following generalization as the simplest law of growth: *Cellular reduplication, once initiated, will continue at a uniform rate in its state of motion until acted upon to change that state.* The closest approach to, and the most familiar example of, this truly ideal mode of augmentation is found during the well-known logarithmic phase of growth so common in bacterial cultures. Various though closely related mathematical expressions describing the course of such unrestricted growth have been widely employed in many investigations, one form representing in plant physiology what is known as Blackman's Law[4] and another constituting the foundation for the extensive analyses of Brody.[5] We prefer, in accordance with the work already reported[1] and with the aid of data from Table I, to write

$$\rho\frac{dq}{dt} = E \qquad (1)$$

where, as formerly, ρ is the resistance of growth, and E the external work done per unit of mass, both ρ and E being considered constant for a given case. The ratio $\left(\frac{E}{\rho}\right)$ thus becomes the "velocity constant of growth" employed by previous writers.

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[4] Blackman, V. H., *Ann. bot.*, 1919, **33**, 353.

[5] Brody, S., Univ. Mo. Agr. Exp. Sta. Res. Bul. 97, 1927.

Experience, however, has also shown quite clearly that this geometric law of increase holds true only during periods that are, in actuality, but a fraction of their respective growth "cycles". Reduplication, and hence growth come sooner or later to a close even though food is just as abundant as before. No known example of growth completely escapes the latter effect, and none proceeds strictly in accordance with the simple expression given in (1). Even where successive tissue-cultures appear to continue their multiplication in geometric fashion, they do so only as a result of artificial intervention, failing which, they too, succumb to this additional and equally important law of decline.

Nor does (1) allow for any growth save that which is ushered in with some positive velocity; it cannot, as is evident, provide for a period of lag, another phenomenon that happens to be notable in bacterial cultures, though it, too, is an intrinsic property of all growing systems. For according to (1) the relative velocity of increase must be just as great at the onset as it is at some later stage of reduplication, whence, if this be zero initially, no growth whatsoever will, or can, take place. Yet there are numerous examples in which growth "takes off from a standing start," and even some in which an initial loss is witnessed.

Thus, to meet the conditions of actual experience we need at least two important amendments to the foregoing basic law of growth, and these are: (1) for the "natural" decline of all finite growth, and (2) for the inertial effects exhibited by many familiar examples. Neither of these amendments, it may be noted, in any way destroys the validity, the generality, or the usefulness of the primary statement set forth above.

But, in addition, growth requires sustenance; it is accompanied by heat production; and it is clearly a phenomenon dependent, as Spencer[6] pointed out, upon energy exchange. We are therefore required to unite all of the foregoing characteristics into some single comprehensive expression with which it will then be possible to investigate the various problems of growth.

This entire set of objectives is effectively and most simply achieved if (1) is amended to read, in terms of work per unit mass,

$$\rho\frac{dq}{dt} = E(t) - \lambda\frac{d^2q}{dt^2} - \frac{q}{\kappa}, \qquad (2)$$

where the agencies for the initiation, $E(t)$, λ, for the potential geometric progression, ρ, $E(t)$, for the decline, κ, are given in the notation already proposed; ρ, λ, and κ being constant in a given case, whereas $E(t)$ will be understood in general to be either constant or, as in the human example, some function of t. These quantities are further described in Table II. Implied in (2), and easily deduced from it with the aid of appropriate substitutions from the table, is the relation defining the connection with concomitant heat production during growth:

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[6] Spencer, H., Principles of Biology, New York, 1898, p. 140.

$$\rho\left(\frac{dq}{dt}\right)^2 + E_c(t)\frac{dq}{dt} + A' = U \qquad (3)$$

where $E_c(t)$ is the work of cellular synthesis per unit of mass, and A' the power per unit of mass required to maintain tissue, which is at rest with respect to the motion of growth, in a state of nutritional equilibrium.

To facilitate reference and to provide a ready check upon the homogeneity of various relations or procedures, Table II contains a synopsis of the more important symbols, dimensions, equivalents, and units of the quantities entering into the equations of motion.

The dimensions of the various quantities are easily determined by using equation (3) as the key; the dynamical equivalents are chosen from the simplest and most appropriate or suggestive physical analogues; and the practical units are tentatively set up by employing both days or seconds as the unit of time though it is realized that one or the other will need ultimately to be selected as the more appropriate unit.

The various steps which have led to the concepts of growth resistance, inertia, permittance, etc., cannot be properly developed here, though their application has been briefly outlined and illustrated in earlier papers.[1] Emphasis is, however, again placed upon the suggestion that information of particular value can be obtained if extraneous conditions such as temperature, $[H^+]$, and quality of nutriment, on the one hand, or if, on the other, substances known "to promote" growth or to act adversely, are examined in respect of their connection with, as well as their effect upon, the fundamental quantities enumerated in the table. To this end studies of growth require not simply measurements of change in size, which give us the *q's* and *z's* of the equations, but they require also a determination of the remaining quantities, especially ρ, λ, κ, and E, that enter just as importantly, if not more so, into the basic processes of growth. As indicated here, and as illustrated previously, the values of these quantities may be computed from suitable data on growth and simultaneous heat production with the aid of equations (2) and (3) or

TABLE II.

*Synopsis of Fundamental Quantities appearing in the Equations of Growth and Metabolism.**

	Name	Symbol	Dimensional Formula	Dynamical Equivalent	Practical Unit
1	Resistance	ρ	L^2T^{-1}	(a) Diffusivity; (b) Action (Quanta) per unit mass	(a) (Cm.)²/Second; (b) Erg Seconds/Gm.
2.	Inductance (Inertia Coefficient)	λ	L^2	Moment of Inertia per unit mass	$(Cm.)^2$
3.	Permittance	κ	$L^{-2}T^2$	Reciprocal Work per unit mass	Kg./Calorie§
4.	Charge or Quantity of Growth	q	Pure Number	Napierian log. of mass	Kg./Kg.
5.	Rate of Growth (Flow of Charge)	$\frac{dq}{dt}$	T^{-1}	Time rate of motion per unit of length	Kg./Kg./Day
6.	Acceleration of Growth	$\frac{d^2q}{dt^2}$	T^{-2}	Rate of change in velocity per unit length	$Kg./Kg./(Day)^2$
7.	Size	z	M	Mass	Kg.
8.	Rate of Gain	$\frac{dz}{dt}$	MT^{-1}	Momentum per unit length	Kg./Day
9.	Acceleration of Gain	$\frac{d^2z}{dt^2}$	MT^{-2}	(a) Force per unit length (b) (Surface Tension)	(a) Dynes/Cm.; (b) $Cal./(Meter)^2$

Energy Supply:				
10. Energy at Source for Growth and Maintenance $\left\{\frac{dS}{dt} = V_s \frac{dq}{dt} + M'\right\}$	S	L^2T^{-2}	Work per unit mass	Cal./Kg.
11. Potential at Source for Growth	V_s†	L^2T^{-2}	Work per unit mass	Cal./Kg.
12. Power at Source for Maintenance	M'	L^2T^{-3}	Power per unit mass	Cal./Kg./Day
Energy Utilization:				
13. Potential in the Cell	V_c†	L^2T^{-2}	Work per unit mass	Cal./Kg.
14. Energy of Synthesis (Proliferation)	E_c†	L^2T^{-2}	Work per unit mass	Cal./Kg.
15. External Work of Growth $\left\{\begin{array}{l} E = E_v - E_c; \\ E_v = V_s - V_c. \end{array}\right\}$**	E†	L^2T^{-2}	Work per unit mass	Cal./Kg.
Heat Production:‡				
16. Maintenance Rate of Heat Production per unit mass	A'	L^2T^{-3}	Power per unit mass	Cal./Kg./Day
17. Rate of Heat Production *per unit mass* (Metabolism), by Equation (3)	U	L^2T^{-3}	Power per unit mass	Cal./Kg./Day
18. Rate of Heat Production ($U \times z$)	$\mathfrak{U}'$	ML^2T^{-3}	Power	Cal./Day
19. Total Heat Output in time t	$\mathfrak{U}$	ML^2T^{-2}	Work	Calories

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* Two quantities, $\mathfrak{F}_s$, and $\mathfrak{F}_c$, the field intensity at source and cell respectively, with the dimension LT^{-2}, are basically implied in this scheme, but they have not as yet been described in connection with the present results.

§ Large Calorie.

† When $E(t)$ in (2) is constant and equal simply to E as defined in item (15). Similarly when $E(t)$ involves t, as in the case of human growth, we have:

$$E(t) = E_v(t) - E_c(t) = [E_v - E_1\epsilon^{-\beta t}\sinh(\theta t - \zeta)] - [E_c + E_2\epsilon^{-\beta t}\sinh(\theta t - \zeta)] = E - E_0\epsilon^{-\beta t}\sinh(\theta t - \zeta);\text{ where, } E_0 = (E_1 + E_2)$$

the dimensions of all the E's remaining the same as in item (15) since the function is and must be transcendental to preserve homogeneity.

‡ Applies solely to basal state.

** The symbol E_v appeared as E_s in previous papers.[1]

their respective integrals. Comparisons made on this fundamental basis will then indicate whether some substance "x", under investigation, has altered growth resistance ρ, inductance λ, permittance κ, or that it has failed to do so.

6787

Physiology of Shigella paradysenteriae var. Sonne.

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From the Iowa State College, Ames, Iowa.

The dissimilation of lactose and sucrose by *Shigella paradysenteriae* var. Sonne has been studied to gain additional knowledge of the phenomenon known as "delayed" or "slow" fermentation. The culture (A.T.C. 31) shows in lactose and sucrose the delayed fermentation characteristic of the Sonne type. In Durham fermentation tubes about 4 days are required for acid formation in lactose broth and about 7 days for sucrose. Colonies on lactose or sucrose agar plates are alkaline or neutral but after a few days develop small secondary colonies as papillae which become acid. If a papilla on sucrose or lactose agar is suspended in sterile broth or saline and plated on agar containing the same sugar, 2 types of colonies appear. One type is acid and does not form papillae even after long incubation, the other type is like the original, alkaline and develops acid papillae. Sugar broth inoculated with the acid type colony is promptly acidified, whereas the alkaline type causes the characteristic delayed fermentation.

Attempts to decrease the time for acid formation in the slow culture by transferring once or twice daily to fresh medium with the same sugar have failed, though this procedure has been carried through 60 transfers. One may easily obtain the rapid variant, however, by plating out a culture which has developed acid, the types of colonies developing on the plates being of 2 types, acid and alkaline, as when the papillae from plate cultures are used.

In order to study the fermentation of lactose and sucrose in more detail than was possible using Durham tubes, flasks have been used, and examination made at least once daily to determine pH, titrable acidity, oxidation-reduction potential, sugar utilized, total population and per cent of population composed of the rapid fermenting variant. In sucrose daily determinations have also been made of formic, acetic, lactic and succinic acids, ethyl alcohol and carbon dioxide.

The typical delayed fermentation of these sugars shows only slight utilization of the carbohydrate until shortly before acidity develops, when the sugar is suddenly attacked. When the culture is aerated 50 to 75% of the sugar may be utilized in a few days, but when not aerated the population remains at lower levels and much less sugar is used.

Following the enhanced utilization of carbohydrate there is an appreciable increase in the total population, and at this time the rapid variant appears. From this time on both types were observed in the cultures, with the rapid variant predominating during the latter phase of the fermentation.

Flasks inoculated with the rapid variant reached a maximum population, greatest utilization of sugar and the medium was acidified on the first day.

6788

Isolation of a Herpes Virus from Several Cases of Epidemic Encephalitis.*

FREDERICK P. GAY and MARGARET HOLDEN.

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

The principal reason that militates against acceptance of the one positive hypothesis of etiology in epidemic encephalitis, namely that it may be due to a neurotropic strain of Herpes virus, is the fact that this easily identifiable virus has been isolated in less than a dozen cases of the disease. The only positive isolations in this country are, so far as we know, those briefly referred to in connection with other work.[1] These first American isolations occurred in 3 successive cases of encephalitis after measles in children. The peculiar conditions explain, we believe, 2 of the reasons why more frequent success has not been obtained by others. In each instance the encephalitis occurred immediately after the exanthem, was clinically charac-

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* This research was supported by the W. J. Matheson Fund for the Study of Encephalitis.

[1] Gay, F. P., Infections of the Central Nervous System, A.R.N. and M.D., 1932, **12**, 191, Williams and Wilkins Co., Baltimore.

teristic, and was histologically consistent with a diagnosis of epidemic encephalitis, but suspensions of these human brains failed to kill rabbits on intracerebral injection. Two of these 3 brains did, however, produce an herpetiform skin eruption in rabbits, proved to be due to a true Herpes virus of a strictly dermotropic type by the fact that immunization with each of these brains by intradermal inoculation in rabbits in several, but not in all instances, rendered these animals insusceptible to inoculation with a known Herpes virus. Another reason for failure is indicated by case number 3 in this series which failed on first inoculation to produce even dermal lesions but became active in this regard when the brain had been aged in glycerine for over a month.[2]

In other words, failure to isolate Herpes virus from encephalitis may be due not only to the scant amount present but also to the fact that an erroneous assumption is made that the virus as obtained from a human case must be neurotropic instead of dermotropic for rabbits.

We have also recently isolated the virus of *Herpes simplex* from 2 acute adult cases of encephalitis which illustrate other reasons for the failure of the majority of observers.

Case 4. A laboratory worker accidentally bitten by a monkey, apparently recovered from the bite, but immediately afterward fell ill of a febrile disease with progressive symptoms of ascending myelitis and died 15 days after the first symptoms of involvement of the central nervous system. The gross and histological picture included areas of softening in the mid brain and widely diffuse areas of perivascular lymphocytic infiltration. The brain of this individual as received was grossly contaminated with a coliform organism but yielded on brain passage through a series of rabbits bacteriologically sterile tissue which killed invariably with characteristic symptoms and which produced an exaggerated skin lesion that was also followed by ascending myelitis and death. The virus present in the brains of these animals was thoroughly identified with known strains of Herpes virus by crossed immunity reactions which will be elsewhere described, and also by producing lethargic encephalitis in Cebus but not in rhesus monkeys.

Case 5 occurred in a male patient aged 27 who had been suffering for 5 years from chronic encephalitis with Parkinsonian syndrome, gyrorotatory spasms of the eye muscles and salivation. Several exacerbations of the acute disease occurred and he was admitted to the hospital in a semi-stupor with a fever of 104° and later fell into coma and died on the 11th day after admission. Gross and histological findings of the brain showed hemorrhagic softening of both dentate nuclei and a generalized perivascular infiltration in many parts of the brain and demyelinization of the white matter of the cortex. Both areas of softening and perivascular infiltration were noted in the basal ganglia.

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[2] Perdrau, J., *Brit. J. Exp. Path.*, 1925, **6**, 123.

These last 2 cases we believe illustrate conditions which must be present but which do not guarantee the findings of a Herpes virus. Brain material, preferably from the region of the basal ganglia, should be used for inoculation; the cases should be in the acute febrile stage, either of the initial acute encephalitis or of an exacerbation of the disease in a chronic case.

We have failed to find Herpes virus in a case of encephalitis following mumps, in another following whooping cough and in another very acute adult case—no one of which differed in any obvious way from these positive cases we have just discussed.

We wish to express our gratitude to Dr. Josephine B. Neal, who has furnished us with the histories and material for all the cases to which reference is made, and to Drs. Orton, Stevenson, and Wolff for the histopathological descriptions.

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Susceptibility of Diabetic Dogs to Tuberculosis.*

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(Introduced by Frederick P. Gay.)

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It has been believed for a long time that diabetes predisposes human beings to infections, including tuberculosis, and that when a diabetic individual develops tuberculosis the disease is extremely severe, and rapidly fatal. The incidence of tuberculosis is almost 3 times as great in diabetics as in the general population (Banyai[1]). Fitz[2] found that 63% of his cases died in less than one year(19 of 31 cases). While most writers agree on these findings (Gotten,[3]

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* This work was made possible by a grant from the Josiah Macy, Jr., Foundation.

[1] Banyai, A. L., *Am. Rev. Tub.*, 1931, **24**, 650.

[2] Fitz, R., *Am. J. Med. Sci.*, 1930, **180**, 192.

[3] Gotten, H. B., *Am. Rev. Tub.*, 1931, **24**, 668.

Adams[4]), they have been disputed by many others. Joslin,[5] and Rosenberg and Wolf[6] feel that the prognosis is not bad in cases of tuberculosis complicated by diabetes. It is, therefore, obvious that much confusion prevails concerning the increased susceptibility of the diabetic to tuberculosis. It was hoped that animal experimentation might help to clarify this problem.

Our experiments include first a study of tuberculosis in the diabetic dog. A series of dogs was pancreatectomized. Eight survived, 7 of which developed severe diabetes while one had only a slight increase in blood sugar. As noted on the accompanying chart the animals survived from 16 to 95 days after infection. The average blood sugar is also included in the chart. Small doses of insulin were given daily to offset coma, and at the same time the animals remained severely diabetic. To replace some of the enzymes of which pancreatectomized animals are deprived, we fed them daily with desiccated pancreatic substance incorporated in their food. Twenty-four hours after operation, the animals were injected intraperitoneally with a human strain of tubercle bacillus (H37) in doses of 1 mg. per pound of weight.

As recorded on the chart, 7 of the 8 animals were macroscopically positive for tuberculosis (87.5%). In 6 of the 7 that have so far been checked histologically the diagnosis was confirmed, while one animal was negative both macroscopically and microscopically. The lungs were more severely involved than the retroperitoneal glands or the liver. The spleen was never found positive on microscopic study. Caseation was encountered only once (in the retroperitoneal glands), and the tuberculosis was never severe. This may be due to the high natural resistance of the animal, or to the fact that the strain of infecting bacilli, which we have kept in our laboratory for the past 10 years, appears to have lost a good deal of its original virulence.

The experiment was controlled with 8 animals. These were laparotomized and closed without interference with the pancreas and infected 24 hours after operation with the same strain (H37), by the same route, and with the same dose. It suffices to say that although 4 of the controls were questionably positive macroscopically, they were all negative on microscopic examination.

In conclusion it appears that the dog is highly refractory to this

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[4] Adams, S. F., *Arch. Int. Med.*, 1927, **37**, 861.

[5] Joslin, E. P., Treatment of Diabetes Mellitus. Lea & Febiger, Phila., 1928.

[6] Rosenberg, N., and Wolf, G., *Klin. Wnschr.*, 1927, **1**, 1935.

TABLE I.
Experimental Results on Susceptibility of Diabetic Dogs to Tuberculosis.

Diabetic Animals Pancreatectomized and Infected							Control Animals Laparotomized and Infected				
			Macro.Path.		Micro.Path.						
No.	No. days surviving infection	Aver. blood sugar: mg./100cc.	+ or —	Organs involved	+ or —	Organs involved	No.	Died or killed	No. days surviving infection	Macro. + or —	Micro. + or —
1	16	130	+	Liver	+	Lungs, liver, mesent. lym. nodes	1	D	9	Lung, liver and spleen?	—
2	29	292	+	Lungs, liver, spleen	+	Lungs,	2	D	13	—	—
3	31	312	+	Lungs, liv., spl., retroper. glands	+	Lungs, retroper. glands	3	D	21	Liver and kidney?	—
4	34	338	—		—		4	K	31	Liver?	—
5	42	349	+	Lungs, liver	+	Liver, retroper. glands	5	D	37	—	—
6	46	314	+	Lungs, liver, spleen			6	K	42	Kidney and liver?	—
7	47	365	+	Lungs, liver	+	Lungs, liver	7	K	46	—	—
8	95	399	+	Lungs, liv., spl., mes. and retro. gl.	+	Liver, retroper. glands	8	K	95	—	—

(All animals infected with H37, intraperitoneally, 1 mg. per lb. weight.)

particular human strain of tubercle bacillus (H37), but becomes definitely susceptible when rendered severely diabetic by pancreatectomy.

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II. Experiments on Immunization with Haptens.

K. LANDSTEINER AND JOHN JACOBS.

From the Laboratories of the Rockefeller Institute for Medical Research, New York City.

Experiments were previously reported[1] confirming the findings of Gonzalez and Armangué that the antigenic properties of alcoholic organ extracts containing Forssman's substance are strikingly increased by adsorption to inorganic adsorbents such as kaolin. Additional evidence for this effect has been advanced by the above authors recently,[2] in particular by establishing that it can be observed with a variety of adsorbents.

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[1] Landsteiner, K., and Jacobs, J., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 570.

To study further the phenomenon discovered by Gonzalez and Armangué, experiments similar to those referred to were carried out with Forssman preparations which had been freed from a large portion of inactive material originally present, by methods described previously.[3] Of the two preparations used, one was easily soluble in water and the other was brought into water solution by the addition of phenol.

It appeared that with these substances practically no antigenicity could be induced by adsorption to kaolin whereas on injecting rabbits with the same preparations mixed with pig serum, all of the animals reacted and the majority produced sera of good titer.

These results suggest the possibility that substances other than the specifically reacting haptens have a part in the reactivation by nonantigenic adsorbents. In this connection it may be mentioned that alcoholic organ extracts (*e. g.*, extracts of horse kidney) prepared by heating, which may be supposed to have a higher content in proteins, are by themselves more apt to induce the production of hemolysins than those prepared at ordinary temperature.*

In experiments with adsorbed bacterial carbohydrates, also, the effect seems to vary with the degree of purity of the substances. Preparations of cholera carbohydrate giving little or no biuret reaction were inactive or only faintly antigenic upon adsorption to charcoal, while preparations giving a strong biuret reaction, though very slightly antigenic themselves (following treatment with alkali), were definitely active in inducing the formation of agglutinins and precipitins in rabbits, after adsorption to charcoal. With purified carbohydrates from Type I pneumococcus and an anthrax-like organism the results were essentially negative, as were those with a nitrogen-free preparation of dextran kindly furnished by Prof. H. Hibbert of McGill University.[4]

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[2] Gonzalez, P., and Armangué, M., and coworkers, *Ier Congres Int. de Microbiologie*, Paris, 1930, **2**, 42; *Comp. Rend. Soc. de Biol.*, 1931, **106**, 1006; 1932, **110**, 217, 220, 223, 216, 228; *Am. J. Hyg.*, 1933, **17**, 277.

[3] Landsteiner, K., and Levene, P. A., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 693; *J. Immunol.*, 1927, **14**, 81.

* The occasional results concerning the stimulating effect of turpentine and trypan blue mentioned in our previous note were obtained with extracts prepared by heating.

That adsorption to inorganic particles may increase the activity of poor antigens was shown in immunization experiments with albumose preparations.[5] Rabbits were injected with a fraction consisting largely of heteroalbumose resulting from peptic digestion of coagulated sheep serum and fractionation with alcohol. Three batches of 5 animals received repeated intraperitoneal injections of 25 mg. each of the preparation, one group receiving the substance without admixture, and the 2 others the same quantity mixed with charcoal and aluminum hydroxide respectively. Whereas in the first batch only one rabbit produced a weakly precipitating antiserum the injection of the material adsorbed either to charcoal or aluminum hydroxide was followed by the appearance of distinctly active precipitins in most of the animals.

6791

Enhanced Action of Morphine in Experimental Nephrosis after Oral Ingestion of Magnesium Sulphate.

ARTHUR D. HIRSCHFELDER. (With the technical assistance of V. G. Haury.)

From the Department of Pharmacology, University of Minnesota.

We have shown[1, 2, 3] that the oral administration of magnesium sulphate raises the plasma magnesium of animals and men with damaged kidneys, but not that of normal individuals.

In view of the old-time clinical teaching that one should exercise caution in the administration of morphine to severe nephritics and to old persons, we have tested the effects of morphine sulphate injections on rabbits whose kidneys were injured by the subcutaneous administration of 10 mg. $HgCl_2$/K. The plasma magnesium of these animals ranged from 1.89 to 2.17 mg. Mg. per 100 cc. and rose to 6.90 to 8.98 mg. Mg. Two hours after the oral administration of 2 gm. per kg. $MgSO_4 \cdot 7H_2O$, 30 mg. morphine sulphate per kilo was administered subcutaneously to 8 of these nephrotic rabbits. In 3 of these narcosis lasted 9 to 11 hours (av. 9⅔ hours) and 5 animals died in deep narcosis in ½ to 5 hours after the injection. The 4 control animals receiving the same dose recovered from the narcosis after 3 to 4 hours. The narcosis of the controls was less intense than that of the nephrotic animals.

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[4] *c. f.* Zozaya, J., *J. Exp. Med.*, 1932, **55**, 325; 1933, **57**, 21.

[5] Landsteiner, K., and van der Scheer, J., Proc. Soc. Exp. Biol. and Med., 1931, **28**, 983.

[1] Hirschfelder, A. D., and Serles, E. R., *J. Clin. Invest.*, 1932, **11**, 841.

[2] Hirschfelder, A. D., and Serles, E. R., *J. Pharmacol. and Exp. Therap.*, 1932, **45**, 264.

[3] Hirschfelder, A. D., (with the technical assistance of V. G. Haury), Proc. Soc. Exp. Biol. and Med., 1933, **30**, 996.

These results indicate that animals, whose plasma magnesium is only moderately elevated (7.0 to 8.70 mg. per 100 cc.) (as in the case in nephrotic patients who have received a single purgative oral dose of Epsom salts), are much more sensitive to morphine than are normal animals. These results are in striking contrast to the results obtained by MacKay and MacKay,[4] who found that "uraemic" (nephrectomized) rats are less sensitive to morphine than are normal rats. The level of the plasma magnesium is most probably the determining factor.

It is therefore evident that morphine should be administered only with the greatest caution to nephritic patients who have received Epsom salts as a purgative. From the results reported with barbitals in the paper to follow it is probable that the same should apply to nephritic patients who have received barbital or phenobarbital.*

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[4] MacKay, E. M., and MacKay, L. L., *J. Pharmacol. and Exp. Therap.*, 1930, **40**, 207.

* This is the more probable because Barbour and Taylor[5] have shown that the administration of $MgCl_2$ subcutaneously increases the rapidity and intensity of barbital narcosis. Although in the doses used by them in normal animals the magnesium salt shortened the duration of narcosis and decreased the toxicity of the barbital it is probable that these latter effects would be somewhat different in nephritic patients where the magnesium could be excreted very slowly.

[5] Barbour, H. G., and Taylor W. F., *J. Pharmacol. and Exp. Therap.*, 1931, **42**, 321.

6792

Effect of Nephrectomy on Duration of Action of Barbitals.

ARTHUR D. HIRSCHFELDER AND VICTOR G. HAURY.*

From the Department of Pharmacology, University of Minnesota.

Fitch and Tatum[1] have divided the barbitals into 2 groups based upon the duration of their action and upon the manner in which they cause death. They found that the longer acting barbitals (especially phenobarbital and barbital, ethyl-n-butyl barbital (neonal), iso-amyl barbital (amytal), ethyl iso-propyl barbital (barbituric acid of ipral), and also iso-propyl bromallyl barbital (noctal) cause death from pulmonary oedema, while the other barbitals, which they group as the short acting barbitals, when injected intraperitoneally in toxic doses cause death from depression.

Our finding that there is a marked difference in action of magnesium sulphate when administered to normal and to nephrectomized rabbits and to nephritic patients[1, 2, 3] led us to try this method of testing barbital action. Nephrectomy in etherized rabbits was done through a lumbar incision and was greatly accelerated by the use of a specially devised kidney fork. This was made from an ordinary table fork the first and third tynes of which were sawed off completely, the second and fourth being sharpened and bent around to form a semicircle of 3 cm. diameter. This was inserted through the lumbar incision so as to grasp the kidney, the renal pedicle passing through the space left by removal of the third tyne of the fork. A plate of spring phosphor bronze 7 cm. long and 2 cm. wide, sliding on a collar surrounding the handle of the fork was then pressed down so as to grasp the kidney completely, the kidney was drawn up through the incision and the renal pedical ligated. The incision

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* This research was rendered possible by a grant of funds by the Hoffmann-LaRoche, Inc. (Basel, Switzerland, and Nutley, N. J.). The drugs used were kindly furnished by the following firms: Allylisopropylbarbituric acid, Hoffmann-LaRoche, Inc.; Pernocton, Riedel-de Haen, Inc.; Pentabarbital, Barbital-sodium and Neonal, Abbot Laboratories; Amytal, Eli Lilly & Co.; Phanodorn and Phenobarbital, Winthrop Chemical Co.; Dial, Ciba Co., and Ipral, E. R. Squibb & Son.

[1] Fitch, R. H., and Tatum, A. L., *J. Pharmacol. and Exp. Therap.*, 1932, **44**, 325.

[2] Hirschfelder, A. D., and Serles, E. R., *J. Clin. Invest.*, 1932, **11**, 841; *J. Pharmacol. and Exp. Therap.*, 1932, **45**, 264.

[3] Hirschfelder, A. D. (with the technical assistance of V. G. Haury), PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 996.

was then sutured. The operation requires 15 to 20 minutes. The animals were allowed to recover completely from the ether anesthetic and the barbital was injected subcutaneously on the following day.

The duration of the anesthesia in nephrectomized animals, as compared with the controls receiving the same dose, gave a much sharper division than Fitch and Tatum's into 2 groups: 1. Barbital and phenobarbital whose narcotic action in nephrectomized rabbits is much longer than in the controls. 2. All the other barbitals tested whose action was scarcely or not at all prolonged in nephrectomized animals as compared with the controls. Barbital gave narcosis averaging 11.5 hours in 7 controls and 79.6 hours in 6 nephrectomized rabbits; phenobarbital, 13 hours in 8 controls and 36.5 hours in 9 nephrectomized. Barbital and phenobarbital narcosis usually lasted till the death of the nephrectomized rabbits.

TABLE I.

	Nephrectomized animals	Control animals
Allylisopropyl (barbital of allonal)	6	6
iso-amyl ethyl (amytal)	7	9
n-butyl ethyl (neonal)	8	9
calcium ethyl isopropyl (ipral)	8	9
di-allyl (dial)	10	10
cyclohexenyl ethyl (phanodorn)	9	9
2-butyl brom ethyl (pernocton)	9	8
ethyl 1-methyl butyl (pentobarbital)	8	8

It is, therefore probable that barbital and phenobarbital are excreted practically unchanged in the urine (as has been shown for barbital by Bachem,[4] Eddy,[5] Reinert[6] and Klingenfuss[7]) and that the other barbitals are broken up in the intermediary metabolism, most probably in the liver.

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[4] Bachem, *Arch. f. Exp. Pathol. u. Pharmakol.*, 1910, **63**, 228.

[5] Eddy, N. B., *J. Pharmacol. and Exp. Therap.*, 1928, **33**, 43.

[6] Reinert, M., *Arch. f. exp. Path. u. Pharmakol.*, 1928, **130**, 49.

[7] Klingenfuss, M., and Reinert, M., *Arch. f. exp. Path. u. Pharmakol.*, 1932, **165**, 416.

6793

Adrenalin Reaction in Pregnancy and in the Preeclamptic State.

JESSE L. CARR. (Introduced by C. L. Connor.)

From the Department of Pathology, University of California.

Variations from the normal physiological response to adrenalin injection, described by Canon and others, have been recorded by many authors. Goetsch[1] demonstrated that in hyperthyroid individuals the response to injection of 0.5 cc. of adrenalin was abnormal, *viz.*, blood pressure rose 10 to 50 points, excessive increase in pulse rate, in respiratory rate, and a rise in the number of leukocytes. Eppinger and Hess[2] found a hyperirritability to adrenalin in some cases of diabetes mellitus. Eppinger, Falta and Rudinger[3] found thyroidectomized dogs and cretins remarkably tolerant to adrenalin when this tolerance was measured by the glycosuric response, while Hare and Karn[4] found women in pregnancy to be less reactive to adrenalin injection than normal women. Louros[5] noted that if the pregnant individual were in eclampsia or preeclamptic state, with an abnormally high blood pressure, that the reaction to adrenalin was entirely reversed.

To repeat Louros' tests on the physiological response of pregnant women in preeclamptic toxemia to adrenalin injection, 1 cc. of adrenalin was given to each of 5 women and their blood pressure, pulse, and respiration noted over the subsequent 2-hour period. All of these women were between the seventh and ninth month of pregnancy, none of them had a blood pressure of over 200, and none of them had had convulsions. They all had a moderate amount of albumen in the urine. After injection and during the period of observation they experienced no excitement or nervousness and felt none of the flushing and stimulation described by normal patients and to a greater degree by those with hyperthyroidism. They uniformly said that they subjectively felt better after than before injection. The blood pressure curves of one normal pregnant woman and 5 women with preeclamptic toxemia are shown below.

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[1] Goetsch, Emil, *New York State J. Med.*, 1918, **18**, 259.

[2] Eppinger, H., and Hess, L., *Z. f. Klin. Med.*, 1909, **67**, 345.

[3] Eppinger, H., Falta, W., and Rudinger, C., *Z. f. Klin. Med.*, 1908, **66**, 1.

[4] Hare, D. C., and Karn, M. N., *Quart. J. Med.*, 1929, **22**, 381.

[5] von Louros, Dr. Nicholas, *Zentralblatt für Gynakologie*, 1923, **47**, 1667.

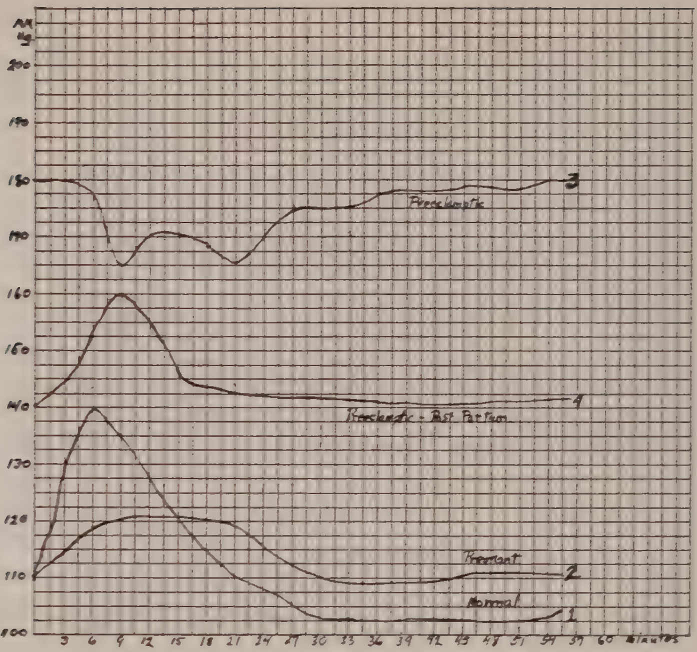

FIG. 1.

Curve 1 shows the normal physiological response to the injection of 1 cc. of 1-1000 adrenalin hydrocyanide hypodermically.

Curve 2 shows a diminished reaction in pregnancy.

Curve 3 is a composite curve of five preeclamptic toxemic women showing a reversed adrenalin reaction.

Curve 4 shows the reaction of a preeclamptic female 6 weeks post partum. The blood pressure is still elevated but the curve has returned to normal.

Conclusion. Women with preeclamptic toxemia show an exaggerated reverse curve when given subcutaneous adrenalin injection.

6794

Relation of Milk Ingestion to Calcium Metabolism in Children.

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From the Iowa Child Welfare Research Station, State University of Iowa.

Calcium, phosphorus and nitrogen retention studies comprising 46 metabolism periods with 8 children between 3 and 5 years of age lead to the conclusion that a pint of milk, furnishing 75% of the calcium, when included as part of a diet containing adequate amounts of phosphorus, protein, fat and vitamins from other sources will supply enough calcium to meet the needs of normal children of the ages considered. The conclusions are based on a comparison of the retentions of these essential growth constituents when the children were taking diets containing a pint and a quart of milk respectively. In one series of tests the children received cod liver oil; in a second, cod liver oil and Viosterol; and in a third during June and July they were given no cod liver oil, but spent a large part of the day out of doors. The average retentions following the various diet modifications are given in the table.

TABLE I.
Calcium, Phosphorus and Nitrogen Retention, gm. per kg., of Children Receiving Different Levels of Milk.

Children No.	Av. Age yr. mo.	Av. Wt. kg.	Diet-Modification Milk cc.	Diet-Modification Vitamin D	Calcium Intake	Calcium Retention	Phosphorus Intake	Phosphorus Retention	Nitrogen Intake	Nitrogen Retention
11	4-4	15.6	475	C.L.O.*	.051	.010	.065	.010	.487	.089
10	4-5	16.3	950	C.L.O.	.082	.010	.079	.008	.50	.071
10	4-6	16.3	475	C.L.O.–V.†	.051	.008	.064	.008	.484	.093
8	4-9	16.7	950	C.L.O.–V	.081	.008	.079	.007	.51	.073
3	4-4	16.6	475	Sunshine	.049	.007	.070	.011	.51	.095
4	4-7	18.0	950	Sunshine	.077	.008	.079	.009	.50	.099

* 10 cc. cod liver oil daily.
† 8 drops Viosterol daily.

Seemingly the difference in the amount of calcium retained by children receiving different levels of milk above a pint reported by other investigators[1] is not related primarily to the variations in the amount of sunshine to which the children were exposed, but to the difference in the physiological condition of the children studied. The data of the study herein reported indicate that less well-nourished children may retain more calcium, phosphorus, and nitrogen during first metabolism periods when a pint of milk and sufficient Vitamin D are included in the diet than during a second successive period when a quart with Vitamin D is given. Well-nourished children retain approximately the same amount of calcium whether receiving a pint or a quart. As will be discussed elsewhere greatly undernourished children may need somewhat more than a pint.

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[1] Sherman, H. C., and Hawley, E., *J. Biol. Chem.*, 1922, **53**, 375; Willard, A. C., and Blunt, K., *J. Biol. Chem.*, 1927, **75**, 251; Wang, C. C., Kaucher, M., and Frank, M., *Am. J. Dis. Child.*, 1928, **35**, 856; Kramer, M. M., Latzke, E., and Shaw, M. M., *J. Biol. Chem.*, 1928, **79**, 283; Stearns, G., Oelke, M. J., and Boyd, J. D., *Am. J. Dis. Child.*, 1931, **42**, 88.

6795

Nitrogen Metabolism in White Mice in the Course of Tumor Development.

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From the Department of Biochemistry, Duke University School of Medicine, Durham, N. C.

Data were presented concerning the composition of the urine of normal white mice.[1, 2] Attention was drawn[2] to the fact that the urine of white mice is remarkably concentrated, average specific gravity 1.068, and to the uniformly high content of a protein of a chondromucoid type.

In the course of a biochemical study of the development of transplanted tumor in white mice studies were made of the composition of the urine and the data compared with that of urine from normal mice. The results obtained are described below.

Three hundred tumor mice and 400 control animals were used. The animals were approximately of the same age and source in both groups, were kept under the same conditions and given the same diet of oats, bread and water.

The tumor material used was Sarcoma 180 of the Crocker Institute. It was transplanted subcutaneously in the usual manner into young adult mice. The virulence of this tumor is uniformly great, producing 95 to 100% takes, and most of the mice die within 6 weeks after the implantation. In most instances by the end of the process the tumors reached relatively enormous proportions with an area of 12 sq. cm., and weighing about one-third of the total body weight. Quite frequently, however, the mice died with much smaller tumors. The total body weight of the mice, including the tumors, remained quite stationary and equal to that of the controls, as shown in Fig. 1a. But the true body weight obtained after excising the tumor declined steadily from the average value of 23 gm. to 16 gm., amounting to a loss of about one-third of tissue substance, which was evidently used for the growth of the tumor.

The urine was obtained directly from the bladder by gentle massage and pooled samples from about 50 animals at a time were analyzed by the methods stated in the previous paper.[2] The tumor mice yielded uniformly less urine than the normal animals. The

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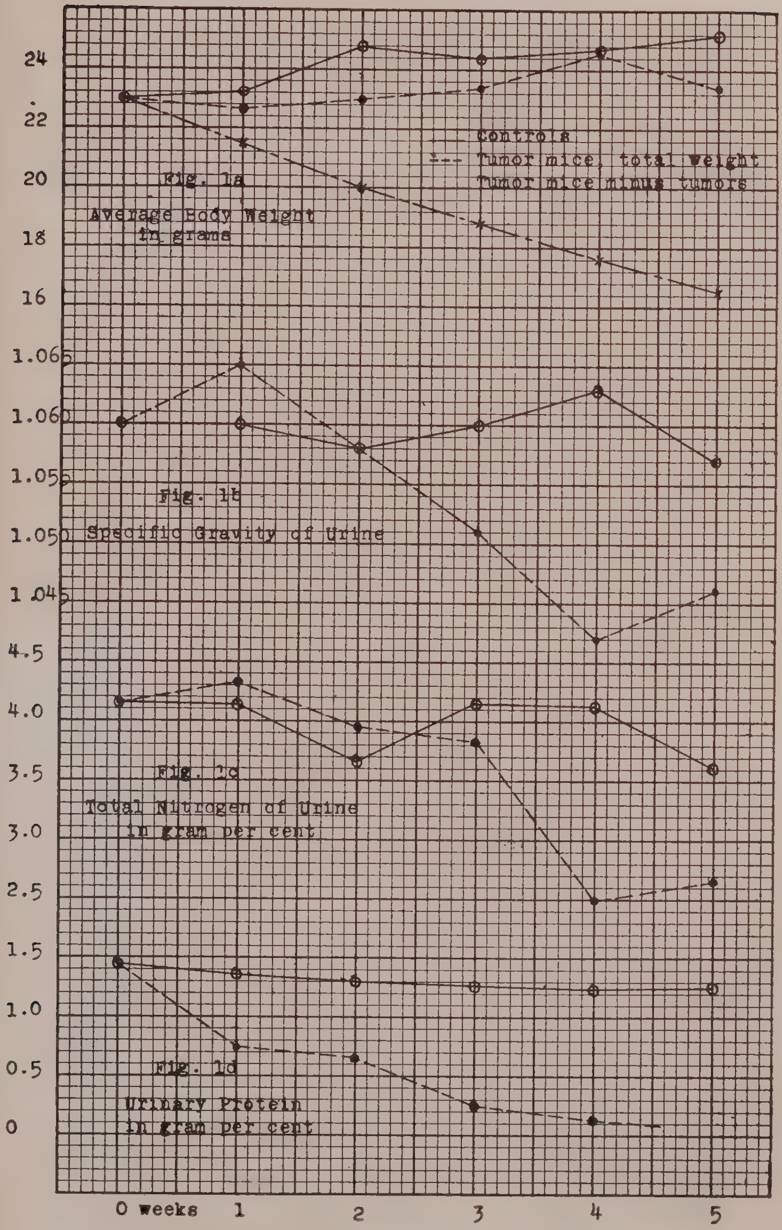
[1] Parfentjev, I. A., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1285.

[2] Parfentjev, I. A., and Perlzweig, W. A., *J. Biol. Chem.*, 1933, **100**, 551.



—— controls; - - - - tumor mice, total weight; —·—·— tumor mice minus tumors.

specific gravity, total nitrogen, protein, urea, allantoin, uric acid, creatine and creatinine, ammonia and amino acid nitrogen and the mineral constituents were determined. Only the first 4 of the above determinations showed significant differences (Figs. 1b, 1c, and 1d) for specific gravity, total nitrogen and protein, respectively. The changes in urea excretion paralleled those of the total nitrogen so closely that they were omitted from the figure.

The specific gravity (Fig. 1b) which in the normal mice varied from 1.065 to 1.057 in this series fell steadily after the first week in the tumor mice to 1.043 in the fourth week and rose somewhat before death.

That the above fall in concentration was due almost entirely to a diminution of urea and of the protein was shown by the decline in the total nitrogen, urea and protein but not in the other constituents, as shown in Figs. 1c and 1d for total nitrogen and protein respectively. During the first 3 weeks after tumor implantation the total urinary nitrogen did not vary from the normal level appreciably, but thereafter it fell abruptly, paralleling the fall in specific gravity. The urea nitrogen fell proportionately, but not the other nitrogenous constituents which remained unchanged.

A part of the decline in the total nitrogen excretion was evidently due to the gradual diminution of the protein content whose steady decrease to mere traces is shown in Fig. 1d.

The figures obtained for ammonia nitrogen and titratable acidity did not show any significant variation from the normal figures indicating the absence of any profound changes in the acid base equilibrium during the growth of the tumor.

It appears quite plausible from the above data that the formation and metabolism of the rapidly growing tumor in the organism of the mouse not only draws largely for its building and energy yielding materials on the body tissues but also attempts to conserve these by a diminished excretion of urea and of the urinary protein. It is attractive to speculate upon the origin and the fate of this chondromucoid-like protein. That it is constantly present in the urine of white mice indicates that it is a normal metabolic product in this animal species. As to its probable fate in the tumor bearing animals no conclusions can be reached at present. A study of the tumor proteins is being carried on with particular attention to the possibility of identifying a protein similar to that of the urine.

These observations are at variance with those of Kimura[8] in white rats with transplanted tumors of the Flexner sarcoma type. Kimura determined the nitrogenous and mineral constituents of the urine and reports an increase in the total nitrogen in the form of urea and allantoin paralleled by an increase in sulfate and phosphate excretion, indicating an increased rate of protein catabolism. Kimura was unable to find any protein and found only a trace of allantoin in normal rat urine, whereas in this laboratory protein has been found as a fairly constant constituent of rat urine and allantoin has been shown to be the predominant end product of purin metabolism in the rat by Hunter, Givens, and Guion.[4]

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[8] Kimura, Y., *J. Biochem.*, 1932, **8**, 469.

Summary. In the course of the growth of Sarcoma 180 in white mice the excretion of nitrogen diminishes chiefly at the expense of urea and of the urinary protein, with the latter tending to disappear entirely. The excretion of the other urinary constituents is practically unaffected.

6796

Formaldehyde Inhibition of Tryptic and Peptic Digestion of Egg White.*

ALDEN K. BOOR.

From the Department of Medicine, the University of Chicago.

In connection with a study of the action of formaldehyde on certain bacterial suspensions,[1] parallel observations were made of its effect on trypsin and pepsin. Little work has been reported on the influence of formaldehyde on tryptic and peptic digestion of proteins. Johannessohn[2] found that preparations of trypsin vary in their susceptibility to this aldehyde; activity of one preparation was unaffected by 20 hours standing with 1% formaldehyde, while that of another was inhibited by 1/20% formaldehyde. He also reported that pepsin was not inactivated by 10% formaldehyde in 24 hours.

In our experiments, however, formaldehyde in low concentrations had a marked inhibitory effect on the action of both trypsin and pepsin on egg-white. Complete inactivation was not accomplished, however, by the presence of 4% formaldehyde as shown by 18 hour digestion. (Table I.)

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[4] Hunter, A., Givens, M. H., and Guion, C. M., *J. Biol. Chem.*, 1914, **18**, 387.

* This work was supported in part by a grant from the A. B. Kuppenheimer Research Fund, University of Chicago.

[1] Miller, C. P., Jr., and Boor, A. K., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 829.

[2] Johannessohn, Fritz, *Biochem. Z.*, 1917, **83**, 28.

TABLE I.

% Formaldehyde During Digestion	cc. N/14 NaOH used in Formol Titration of Products of Egg White Digestion with	
	Pepsin	Trypsin
Control	8.00	10.00
.004	8.00	8.00
.04	8.00	7.70
.02	6.50	.95
.4	2.63	.55
.8	2.00	.35
2.0	1.80	.20
4.0	.50	.04
8.0	0	0

Allowance has been made for control protein subjected to same conditions of temperature and hydrogen ion concentration for the period of digestion.

Two solutions of raw egg white containing 0.25% and 2.5% protein respectively and 0.4% trypsin* were divided into 10 cc. volumes, and each series treated with varying concentrations of formaldehyde. The reaction was adjusted to pH 7.4 and the mixtures allowed to digest at 37-40° for 18 hours. Similar concentrations of macerated coagulated egg white were digested with 0.1% pepsin at pH 2.5. At the end of the digestion period the liquids were neutralized and the extent of digestion determined by means of Sorensen's formol titration method.

The results in the case of the series containing 2.5% egg white are given in the accompanying table.

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* A commercial product of high grade sold under the label "Special Trypsin" by the Wilson Laboratories, Chicago. The pepsin from the same firm is designated of 1:10.000 potency.

6797

A Note on the Mechanism of Fixation in an Area of Sterile Inflammation.*

VALY MENKIN.

From the Department of Pathology, Harvard University Medical School.

In explaining fixation of various foreign substances at the site of inflammation as the result of mechanical obstruction by thrombosed lymphatics and by a fibrin network in tissue spaces of the affected area, 3 types of evidence were presented: (1) the appearance of thrombosed lymphatics and of a fibrinous network; (2) the inability of substances to enter the site of inflammation when injected at its periphery; (3) finally, the inhibition of fixation upon the addition to the inflammatory irritant of a fibrin solvent, concentrated urea.[1, 2, 3] It has been pointed out that both factors, coagulated plasma in tissue spaces and fibrinous clots occluding lymphatics at the site of inflammation, can well account for fixation. Such clots in lymphatics have been seen *in vivo* as well as in fixed preparations.[4] At no time, however, has the writer maintained that either one of these 2 factors was more responsible for fixation than the other. In the types of inflammation studied previously with aleuronat or croton oil, both factors, *i. e.*, a fibrinous network in tissue spaces and lymphatics occluded by thrombi had been found. This, though, does not preclude in the least that with some types of inflammatory irritants, fixation may not be predominantly due to one of these 2 factors.

Recently Field, Drinker and White[5] on the basis of the large amounts and increased pressure of free flowing lymph obtained from the main lymphatic trunk of the hind ankle in the anesthetized dog some hours after immersion of the paw in boiling water, conclude that it is more reasonable to ascribe the fixation of trypan blue to its inability to diffuse through the "gelatinous"-appearing subcutaneous tissue which was observed in such inflamed areas rather than to generalized thrombosis of lymphatics.

The view as expressed by these investigators is difficult to accept

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* This study was aided by a grant from the DeLamar Mobile Research Fund.

[1] Menkin, V., *Arch. Path.*, 1931, **12**, 802.

[2] Menkin, V., *J. Exp. Med.*, 1931, **53**, 171.

[3] Menkin, V., *J. Exp. Med.*, 1932, **56**, 157.

[4] Menkin, V., *J. Exp. Med.*, 1931, **53**, 179.

[5] Field, M. E., Drinker, C. K., and White, J. C., *J. Exp. Med.*, 1932, **56**, 363.

for several reasons. In the first place the measurements on lymph pressure obtained from a main lymphatic trunk at the ankle do not indicate the status of all the lymphatics throughout so extensive an area of injury as that produced by immersion of a paw in boiling water. The possible presence of occluded lymphatics in the superficial more intensely affected layers of tissue in which trypan blue was injected is not ruled out by the finding of an increase in the lymph flow and a rise in the pressure of a main lymphatic trunk. As has been recently pointed out by Hudack and McMaster,[6] it is to be noted that when the degree of damage is relatively mild, the drainage through the lymphatics is increased. Consequently the simultaneous occurrence of increased lymph pressure in a main lymphatic trunk draining a considerable area injured to an unequal degree and the fixation of trypan blue at its point of injection which is situated in a relatively small superficial area of presumably lethal damage does not preclude the possibility of local thrombosed lymphatics as the cause for the fixation of the dye. In brief, the measurements as recorded by these investigators do not indicate the status of all the lymphatics at the site of trypan blue inoculation. The writer has pointed out previously[2] that additional information is desired on the relation between exudation from blood vessels and changes in flow of lymph from the inflamed area. Such information is not provided in the study of Field, Drinker and White. An absolute increase in lymph flow in a main lymphatic trunk does not necessarily prove that there may not be a relative fall in the lymph flow if the increased amount of fluid passing out through the vascular capillary wall in an inflamed area is taken into consideration in evaluating changes of flow in the lymphatic vessels with inflammation.

In the second place the term "gelatinous" as used by Field, Drinker, and White in describing the inflamed subcutaneous tissue is not quite clear unless it refers to the gross physical appearance of coagulated serum in the injured area which on microscopic examination is seen to be composed of a fibrinous reticulum. If the term refers solely to the edematous fluid held in tissue spaces acting as a mechanical barrier, then it is difficult to explain all of the writer's previous findings when the peritoneal cavity was chosen as the site of inflammation and aleuronat as the irritant. This irritant as a rule yielded scarcely any edematous fluid in the peritoneal cavity. Yet foreign substances injected into the inflamed cavity were fixed and the retrosternal lymphatics were found to be thrombosed.

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[6] Hudack, S. S., and McMaster, P. D., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 944.

In the third place, Field, Drinker, and White reported no histological examination of tissue subjected to water at 100°C. Precise information as to what structural changes have occurred at the site of inflammation can be readily obtained by microscopic study.

For these 3 reasons similar studies were performed on the forelimb of rabbits. This animal was chosen since the writer's previous experiments had been performed on either the rabbit or the frog.

Under ether anesthesia the foreleg of a rabbit was dipped for about 2 minutes in water heated to 100°C. The animal was allowed to come out of the anesthetic. After a variable interval of time 3 cc. of 1% trypan blue in saline was injected subcutaneously into the inflamed foreleg and into the opposite foreleg to act as control. The axillary lymph node and lymph drawn from the efferent lymphatic vessel in each foreleg were studied for the presence of the dye. In one animal (Rabbit 10-50) both forelegs were dipped into water at 100°C. inasmuch as a sufficient number of controls had been reported in previous studies.[7] The degree of swelling was unusually extensive as compared with that obtained with other types of inflammatory irritants. Some areas of the skin were distinctly discolored with evident patches of necrotic tissue surrounded by a zone of erythema. The swelling extended well into the axilla and in some cases over the anterior chest wall. At the conclusion of an experiment the inflamed tissue was removed and placed in 10% formaldehyde for subsequent histological examination.

The results of these experiments appear in Table I. When trypan blue is injected only several hours after dipping the foreleg in water at about 100°C. no definite evidence of fixation is obtained in spite of a prominent gelatinous-like exudate in the injured area. Microscopic examination of such inflamed areas reveals considerable edema, dilatation, and congestion of small vessels and a very slight cellular infiltration. There is no fibrinous network and the lymphatic lumina are unoccluded and somewhat dilated. The microscopic picture is entirely in accord with the findings, *i. e.*, in regard to the prompt diffusibility of the dye to the tributary lymphatics. These results in the early stage of the inflammation are contrary to the statements of Field, Drinker, and White, claiming that the dye was fixed. This discrepancy may perhaps be ascribed to the different type of animal used and possibly to the fact that, as indicated in the communication of these investigators, the injection of the dye and the readings concerning its drainage into the lymph channel were made while the animal was inactive and under general anesthesia. This was not the case in any of the writer's experiments; the anesthesia was discontinued immediately after exposing the limb of the

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[7] Menkin, V., *J. Exp. Med.*, 1929, **50**, 171.

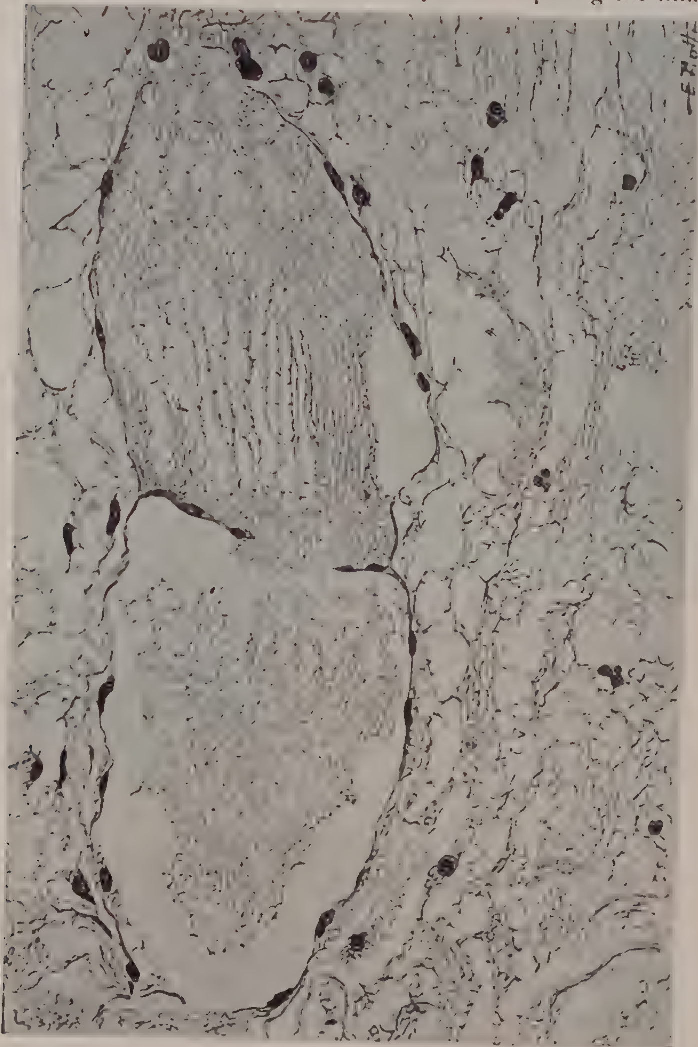

FIG. 1.

Drawing of a lymphatic vessel in an area of cutaneous inflammation of about 22 hours' duration. The inflammatory reaction was induced by water heated to 100°C. The lumen is occluded by a delicate fibrinous reticulum. (Rabbit No. 10-50.) Magnification about 500.

TABLE I.

Retention of Trypan Blue at the Site of Inflamed Areas Induced by Water Heated to 100°C.

Rabbit No.	Interval between exposure to irritant and injection of dye Hr. Min.	Total duration of inflammation Hr. Min.	Presence of dye on inflamed side		Presence of dye on normal side	
			Lymph of efferent lymphatic	Lymph node	Lymph of efferent lymphatic	Lymph node
7-10	4:55	7:10	+	+	++	+
7-03	5:35	8:25		+		+
10-50	19:35	22:15	0	0		
			0	0		
6-25	18:11	21:18	0	FT	++	++
7-11	47:05	50:15	T	0	+	++

T = trace. FT = faint trace.

animal for about 2 minutes in hot water. It is quite conceivable that a dye injected and studied while an animal is under general anesthesia may be delayed from reaching readily lymphatic channels.

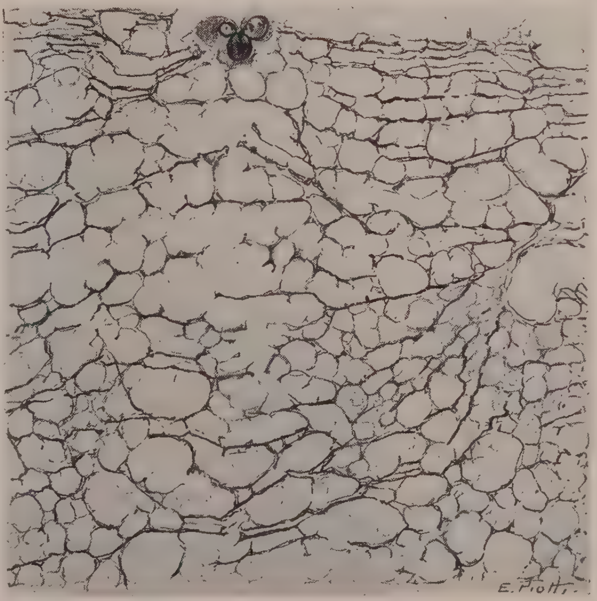

FIG. 2.

Fibrinous meshwork in an area of inflammation of about 22 hours' duration. The inflammatory reaction was induced by water heated to 100°C. Trypan blue injected into this area failed to reach the tributary lymphatic nodes. (Rabbit No. 10-50.) Magnification about 1200.

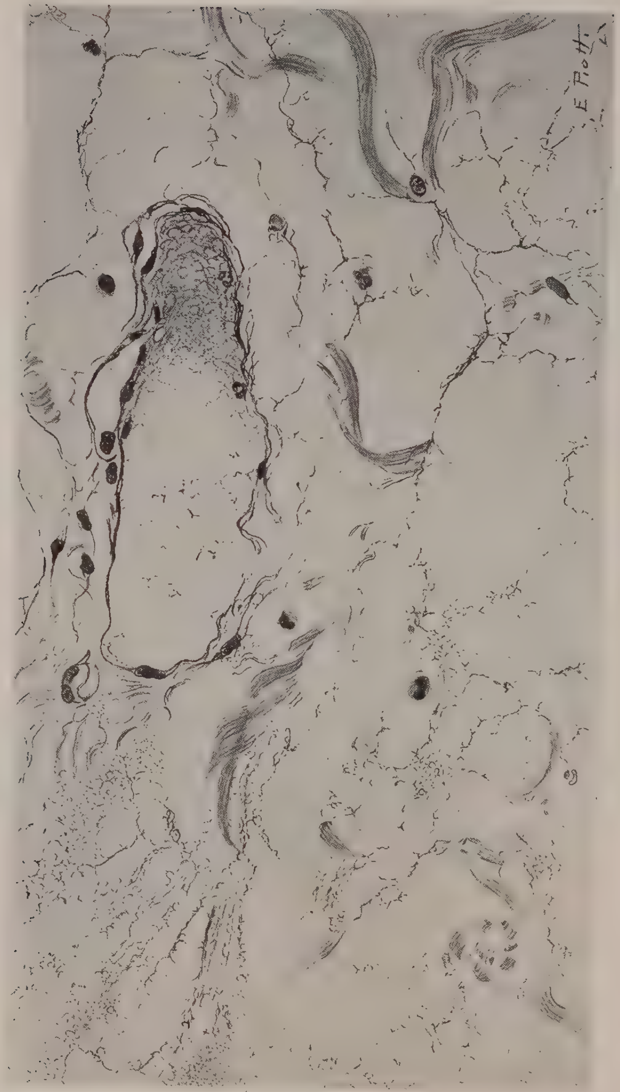

FIG. 3.

Drawing of a lymphatic lumen from an area of inflammation of about 22 hours' duration. The reaction was induced by water heated to 100°C. The lumen is only partially occluded by a delicate fibrinous thrombus. (Rabbit No. 10-50.) Magnification about 500.

owing to muscular atonicity. Mere handling of the leg might under such circumstances favor the passage of the dye into the lymph. However, a retardation of a dye at the site of inflammation in such an abnormal condition as inactivity under general anesthesia cannot certainly be regarded as fixation in the way that the author's previous studies have demonstrated.

The results in Table I show that fixation of trypan blue was obtained only 18 to 19 hours after exposure of the foreleg to hot water. Microscopic examination shows at this stage a very extensive fibrinous network in tissue distended by considerable edema (Fig. 2, Rabbit 10-50). Many lymphatic vessels are found. Some lumina are practically entirely occluded by a fine fibrinous reticulum (Fig. 1, Rabbit, 10-50); others, however, show only partial occlusion by a small fibrinous thrombi (Fig. 3, Rabbit 10-50) and finally, some lymphatic vessels are perfectly patent. These observations are in accord with the explanation that fixation of the dye is due, as in other types of inflammation, to the presence of mechanical obstruction in the form of a fibrinous network in tissues distended with edema and to the occlusion of some of the lymphatics by fibrinous thrombi. It is possible that in these types of inflammation the fibrinous network is of greater consequence than the thrombosis of lymphatics in permitting fixation to take place. However, this fact does not change the basic explanation for the mechanism of fixation. The two factors play the dominant part in mechanically arresting the free diffusion of foreign substances from the site of inflammation. That in some varieties of inflammatory reactions one factor may play a more prominent rôle than the other is quite obvious. The intensity of fixation is probably a function of the number of occluded lymphatics at a given time as well as of the presence of the fibrinous meshwork. As long, however, as some lymphatics vessels are patent, an absolute increase in lymph flow and pressure is to be expected in a main channel draining a large area of edematous swelling. As the inflammatory reaction progresses and more lymphatics become occluded, the lymph flow is likely to diminish. This seems to be precisely the case found by Field, Drinker, and White, as seen from their statement: "One observation was made on the inflamed leg 24 hours after the onset of inflammation. In this case the lymph was still flowing though not as abundantly as it had been 12 hours previously".[5]

Concentrated urea was shown[3] to dissolve fibrin and in this manner to prevent fixation. The foreleg of each of 2 rabbits was exposed to boiling water for about 2 to 3 minutes, followed immediately by subcutaneous injection in that limb of 7 cc. of 50% urea in distilled water. About 18 hours later the injured leg revealed a tremendous amount of edema and congestion. Trypan blue was injected into the injured and normal forelimbs as described above. Two to 3 hours later the dye was seen to have penetrated to the regional lymphatics on the inflamed side as readily as under normal circumstances. The results reported in this communication are in agreement with previous observations and indicate that fixation at the site of a sterile inflammation induced by hot water is due to mechanical obstruction in the form of a fibrinous network, *i. e.*, coagulated plasma, and in part to the presence of a number of lymphatics that are occluded by fibrinous thrombi.

6798

Configuration and Anesthetic Activity of Aromatic Alcohols.*

P. K. KNOEFEL† AND G. A. ALLES. (Introduced by C. D. Leake.)

From the Pharmacological Laboratory, University of California Medical School, San Francisco, and the Laboratory of George Piness, M.D., Los Angeles.

In general, local anesthetics are deficient in duration of action rather than potency. A striking demonstration of prolongation of effect as a result of a small change in structure of a drug-compound has recently been afforded. It is definitely established in the sympathomimetic amines that the transformation of the side chain so that the carbon bearing the nitrogen becomes a secondary carbon atom results in prolongation of effect.[1] To test the validity of this principle in another field, we are now studying for local anesthetic activity some derivatives of ephedrine in which the same peculiarity of structure exists. Of other series, procaine type compounds do not readily lend themselves for this study because of the difficulty of preparing the desired amino-alcohols. However, the aromatic alcohols with pertinent structure are easily obtained. Some of these have been studied before; in fact it has been stated,[2] on rather invalid evidence, that the change from primary to secondary to tertiary alcohol reduces the activity.

Anesthesia of the rabbit cornea and the frog sciatic nerve has been determined for a series of primary, secondary, and tertiary alcohols,

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* Supported in part by the Christine Breon Fund for Medical Research.

† Fellow of the National Research Council.

[1] Alles, G. A., *J. Pharmacol. Exp. Therap.*, 1933, **47**, 339.

[2] Hirschfelder, A. D., and Bieter, R. N., *Physiol. Rev.*, 1932, **12**, 190.

TABLE I.
Anesthetic Effects of Certain Aromatic Alcohols.

Compound and Formula	Boiling Point °C/10 mm.	Duration Anesthesia Rabbit Cornea with Saturated Sol. $<$ 0.1M	Onset of Anesthesia Frog Sciatic Nerve with Concentration:	
			0.05 M	0.04 M
		min.	min.	min.
Phenyl carbinol: $C_6H_5CH_2OH$	92	2*	14	24
Methyl phenyl carbinol: $C_6H_5CH(CH_3)OH$	93-4	4*	7	10
Ethyl phenyl carbinol: $C_6H_5CH(C_2H_5)OH$	102-4	10	5	6
Dimethyl phenyl carbinol: $C_6H_5C(CH_3)_2OH$	89-91	8	9	10
Benzyl carbinol: $C_6H_5CH_2CH_2OH$	100	5	7	10
Methyl benzyl carbinol: $C_6H_5CH_2CH(CH_3)OH$	99-100	10		8
Dimethyl benzyl carbinol: $C_6H_5CH_2C(CH_3)_2OH$	96	2		16
Phenyl ethyl carbinol: $C_6H_5CH_2CH_2CH_2OH$	117-8	5		11
Phenyl vinyl carbinol: $C_6H_5CH{=}CHCH_2OH$	134	2		13

* 0.1 Molar.

all of which were redistilled before use. The results from local application of aqueous solutions at the concentrations noted are seen in the table in which the figures indicate average duration of anesthesia in minutes for the cornea in 3 different rabbits for each drug, and average time in minutes for the production of sensory block of the sciatic nerve in at least 4 different frogs in each instance. Tests were made at minute intervals and the respective findings usually agreed within 2 minutes. The data indicate the relative merits of the compounds both with regard to duration of anesthesia and speed with which it may be induced.

It appears that increase in the length of the straight carbon chain and/or the transformation to secondary and tertiary alcohol may increase the activity. The presence of unsaturation seems undesirable. The expectation is fulfilled that the secondary alcohols are more active than the primary.

6799

Sex Reversal in Amblystoma. VI. Interactions of Ovary and Testis in Arkansas Strain of Amblystoma Punctatum.*

R. R. HUMPHREY.

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In experiments in which one gonadic preprimordium of an embryo was replaced by that of another, it was found that in the local (Buffalo, N. Y.) strain of *A. punctatum* an ovary developing in the presence of a testis was commonly modified under its influence and eventually reduced to the so-called freemartin condition.[1] In such an ovary the formation of the ovarial sacs (central cavity) is suppressed and the growth of the cortex is inhibited; the gonad is thus eventually reduced to a rudimentary or atrophic structure in which only a few oögonia remain. A comparable modification of the ovaries has been induced in females of this strain joined in parabiosis with males.[2]

In the experiment here reported, transplants of the gonadic preprimordium were made in embryos of this same species but of a local race or strain secured from Arkansas. In this strain the ovaries of ungrafted females were found to develop normally, while the gonads of the males commonly exhibited a more pronounced bisexual or hermaphroditic condition than is usually encountered in males of this species. In many cases the gonad had the structure of a testis in its caudal portion, with its cephalic end tending to resemble an ovary (Figs. 1 and 2), but in even more cases the bisexuality consisted rather in a pronounced development of the covering germinal epithelium to give a prominent 'male cortex' over a large part of the length of the gonad (Fig. 3). In males under 4 months of age this cortex was sometimes found to be hypertrophied, with many of its germ cells in the spireme stages characteristic of early oöcytes; in such gonads the medullary or testicular portion was either poorly developed or possessed cavities of greater or less extent (comparable to those of ovarial sacs) in which numerous spermatogonia lay free, often in some phase of degeneration (Fig. 4). With further extension of these cavities and degeneration of the spermatogonia con-

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* This study has been aided by a grant from the Committee for Research in Problems of Sex of The National Research Council.

[1] Humphrey, R. R., *J. Exp. Zool.*, 1929, **53**, 171.

[2] Humphrey, R. R., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 713.

tained in them, the gonad became transformed into an ovary (Fig. 5), which, after further development of its cortex and the final disappearance of the spermatogonia, became indistinguishable from the ovaries of zygotic females. It was concluded that in all about 40% of the zygotic males underwent transformation into females.

Despite the bisexual or hermaphroditic character of the testes in this strain and their tendency to transform into ovaries as above described, it was found that in the grafted animals an ovary in the presence of a testis was inhibited and modified thereby in 25 out of 59 possible cases (42%). Of the 16 animals of this group killed at 2 to 5 months of age, only 8 (50%) showed enlarged oöcytes (auxocytes) in the modified ovary as in Figs. 6 and 7. These cells probably would have disappeared eventually, since many could be found undergoing cytolysis. In 6 of these older animals (38%) no large oöcytes were to be found in the modified ovary, while in 2 (12%) only oögonia remained (Figs. 8 and 9).

In 34 out of 59 possible cases (58%) the ovary developed in the presence of a testis was either normal or but little modified (Fig. 10) while the associated testis (either that of graft or host) showed more pronounced modification (Fig. 11) or even a more or less complete transformation into an ovary (Fig. 12). That such reversal was in part dependent upon the influence of the associated ovary is indicated by the fact that it occurred more frequently here than in ungrafted animals (58% of all possible cases as compared with 40% in ungrafted animals).

It is possible that the 'spontaneous' reversal of males noted above may have been due to the low temperature at which the animals were reared, since Witschi states that development of the medullary portion of the gonad in Amblystoma is delayed by low temperature, which thus favors the growth of the cortex and the differentiation of the gonad as an ovary.[3] In any event, it would appear that the zygotic males whose gonads transformed into ovaries did not later revert to males in the manner described by Witschi for the grass frog, since 70% of the animals over 4 months of age proved to be females, whereas among the animals of 2 to 3 months, approximately 50% could be classified as males either normal or undergoing reversal.

It must be emphasized that no gonad in animals of this strain was found to develop first as a typical ovary, with subsequent transform-

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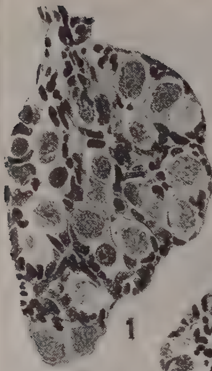
[3] Witschi, E., Sex and Internal Secretions, 1932, Chapter V, Williams and Wilkins, Baltimore.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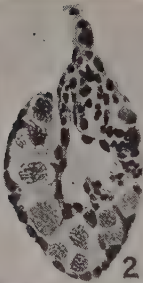
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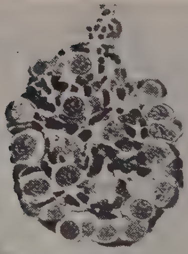
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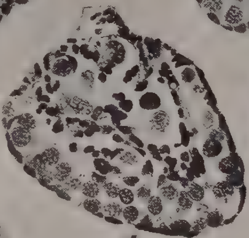
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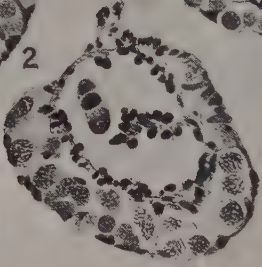
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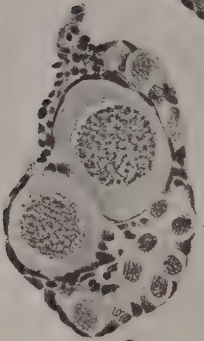
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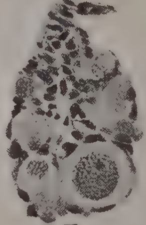
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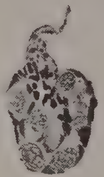
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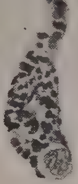
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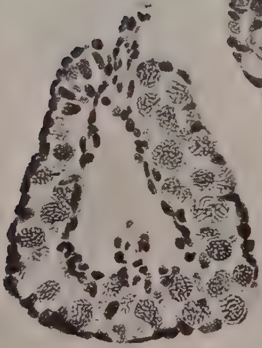
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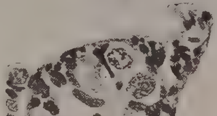
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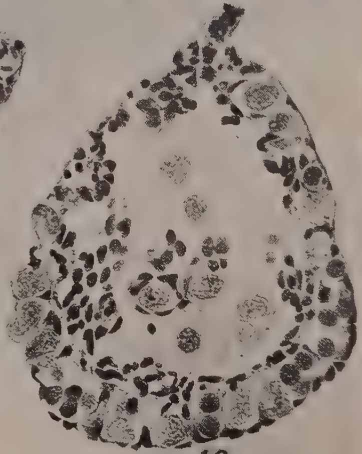
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All figures are photographs of transections of gonads, × 170.

Fig. 1. Caudal end of bisexual gonad, male of 110 days.
Fig. 2. Cephalic end of gonad shown in Fig. 1.
Fig. 3. Testis with prominent cortex, male of 80 days.
Fig. 4. Early cavity formation with degeneration of spermatogonia, male of 80 days.
Fig. 5. Same gonad as in Fig. 4, but showing a region with fewer spermatogonia and cortex more advanced.
Fig. 6. Modified ovary, in male host of 140 days.
Fig. 7. Modified ovary of female carrying a testis graft. 109 days.
Fig. 8. Modified ovary of female carrying a testis graft. 148 days.
Fig. 9. Graft ovary, freemartin type, from male host of 148 days.
Fig. 10. Graft ovary of normal structure in male host of 80 days.
Fig. 11. Modified testis of host carrying the graft ovary shown in Fig. 10.
Fig. 12. Testis in reversal, from a host of 110 days, carrying a graft ovary. Note degenerating spermatogonia in central cavity.

ation to a testis, as is seen in the so-called undifferentiated races of frogs. After the onset of sex-differentiation, the gonads of zygotic males were generally found to be of testicular structure in a large part of their extent. When the medullary (testicular) component of such a bisexual gonad was able to inhibit the hypertrophy of the ovarian component (either cephalic ovarian portion, or continuous 'male cortex') and so escape reversal, it was commonly able to inhibit and modify the growth of an ovary developing with it in the same animal or reduce that ovary to a freemartin condition. It would therefore seem probable that the testicular substance ('medullarian') antagonistic to cortical (ovarian) growth and differentiation was released into the blood stream as well as transferred by diffusion into the adjacent cortex of the bisexual gonad.

The reduction of the ovary to a freemartin type in a considerable proportion of cases in the present experiment is in contrast with the results obtained by Witschi in an Ozark Mountain (Arkansas) strain of *A. punctatum.*[4] Witschi states that "in heterosexual parabiotic twins of this race the gonads of the female mate develop almost normally (no freemartin effect in this case)". Whether this is due to the decreased intensity of the interaction of the gonads in parabiotic twins as compared with grafted animals, or whether the animals used by Witschi are actually of a different local race than that studied by the writer can not be stated. My findings, however, support Witschi's conclusion that "sex antagonism is low in forms that constitutionally are inclined toward hermaphrodism," since they indicate the action of the testis upon the ovary to be either late in onset or of relatively low intensity.

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[4] Witschi, E., *Anat. Rec.*, 1932, **54**, 76.

6800

An Entero Depressant Factor in Stools of Monkeys Infected with Experimental Poliomyelitis.

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Previously, one of the authors (J. A. T.) had demonstrated what was thought to be a toxic factor in the stools of patients ill with poliomyelitis.[1] Was this factor also present in animals experimentally infected with poliomyelitis virus?

Stool specimens were first collected from 10 *Macacus rhesus* monkeys, and the animals were then injected, under complete anesthesia, intracerebrally with potent poliomyelitis virus. Stools were again collected after poliomyelitis had set in. The specimens of feces were dried *in vacuo,* weighed, ground and enough normal saline solution was added to the powdered feces to make a 10 to 20% suspension. This was then passed through a linen cloth to remove gross particles and debris.

Young rabbits weighing 1 to 1½ pounds were etherized, the spinal cord and connections were severed in the region of the tenth thoracic segment, an opening was made, under complete anesthesia, into the peritoneal cavity as described by Sollmann,[2] the intestines were exposed, a cannula connected with a Marriotte bottle attached to the small intestine about 6 inches from the ileocecal valve and the material was introduced into the lumen of the intestine in quantities of 10 cc. This procedure gave a partial spinal animal with nerves and blood vessels intact as far as the piece of gut experimented upon was concerned. The pouch was filled with liquid paraffine at 38°C. The intestinal activity was recorded by a lever connected with the intestinal wall by a silk thread attached to a serrefine.

Ten of the 10 stool suspensions obtained before the injection of the poliomyelitis virus acted as irritants and caused an increase in the number and strength of peristaltic waves. One of the injected animals did not contract poliomyelitis. Every stool suspension obtained from the 9 remaining animals either a few days after paralysis or at postmortem depressed the intestine. This intestinal depression was so complete in 8 of the 9 specimens experimented with, that barium chloride, even in solutions of 1/1000, had no effect on the intestine.

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[1] Toomey, John A., *J. Prev. Med.,* 1932, **6**, 379.

[2] Sollmann, Torald, *Am. J. Physiol.,* 1923, **63**, 395.

Two per cent suspensions of fresh poliomyelitis virus in normal saline solution also caused the same depressant action on the intestines of each of the 6 animals tested.

6801

Agglutinin Titer Value Changes in Blood Serums of Monkeys Experimentally Infected with Poliomyelitis Virus.*

JOHN A. TOOMEY.

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Blood serum of human beings convalescent from poliomyelitis has a higher agglutinin antibody titer for various enteric organisms than has human serum that is taken from the same patient at the onset of the illness.[1] Was this increased titer found in convalescence a new acquisition and the titer found at the height of the disease the normal titer level, or was the titer in convalescence merely a return to a normal level after a temporary decrease that occurred at the height of the disease?

The normal blood serums of twelve 4 to 8 pound *Macacus rhesus* monkeys were tested and found to be rarely higher than 1:80 in agglutinin titer for the several enteric organisms used (*B. typhoid,* paratyphoid A and B and various *B. coli* organisms). The monkeys were then injected with a mixed vaccine of killed typho-coli-paratyphoid organisms in increasing doses together with a mixture of toxins produced by growing the various organisms in broth for 10 days. These injections were given in small doses at intervals of from 4 to 7 days for a period of about 42 days.

After a rest interval, the blood serums were tested and were found to have an agglutinin titer of 1:640 at least and occasionally a titer of 1:2560 for the various enteric organisms used.

The animals were then injected intracerebrally with virulent poliomyelitis virus. It was found that with the advent of paralysis in the

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* Expenses Defrayed in Part by a Grant from the Marion R. Spellman Fund, The Cleveland Foundation.

[1] Toomey, John A., in preparation.

animal the enteric agglutinin titer dropped sharply; with complete prostration and palsy, the titer value was reduced to 1:10 values and often to zero.

6802

Relationship Between Chloride Content and Blood Cerebrospinal Fluid Bromide Ratio.

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From the Iowa State Psychopathic Hospital, Iowa City.

In previous investigations attempts were made to determine whether there were any relationships between the amounts of various substances contained in the blood and cerebrospinal fluid and the distribution ratio in these two fluids of bromide administered and determined by the Walter method. Of these substances, the chloride salts, by virtue of the very close relation that exists between them and bromides, were the first to attract our attention in this respect. It has been shown that the blood chloride content is lower than that of the cerebrospinal fluid (the ratio being about 0.80 to 1). Furthermore, in the cases of mental disease that we have been investigating, this ratio fluctuates within narrow limits. Our first investigation, then, was to determine whether there was any direct relation between these fluctuations and that of the bromide distribution. Such a relation could not be found to exist. In a series of cases of manic depressive psychosis we found a variation of the chloride ratio between 0.79 and 0.89, but this fluctuation did not bear any direct relationship to the changes in the bromide ratio.[1]

The technique of our method of determination of the bromide ratio, however, introduced a new factor, the fact that the blood serum had to be diluted with 2 parts of water before it could be compared with the spinal fluid, since the bromide content of the blood is usually about 3 times that of the spinal fluid. In previous investigations we have used distilled water for this purpose, but this meant that the chloride content of the diluted serum was brought down to one-third of its original content. The blood chloride content is usually about 0.6%, and the question arose, whether the reduction of this to about 0.2% did not influence the bromide ratio obtained. With this in mind we undertook a series of double determinations in 32 cases. In each of these cases we first obtained the quotient (*i. e.,* blood bromide content: cerebrospinal fluid content) by diluting the blood serum with 2 volumes of distilled water, and then using part of the same specimen, we diluted the serum with 0.65 NaCl. The results are represented in Chart 1.

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[1] Rothschild, D., and Malamud, Wm., *Arch. Neurol. and Psychiat.*, 1931, **26**, 829.

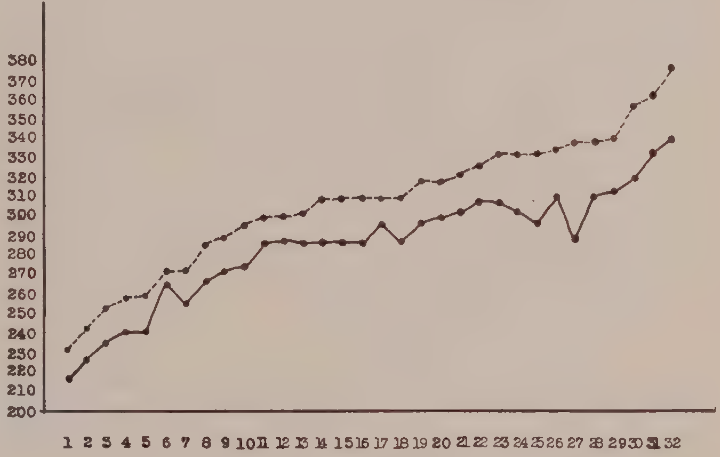

CHART 1.

The figures at the bottom represent the numbers of cases. The figures at the left hand margin are the quotients from 2.00 to 3.80. The dots connected by the continuous line represent the quotients obtained by using the 0.6% NaCl solution to dilute the serum. The dots connected by the interrupted line represent the quotients obtained in the same cases where distilled water was used for the dilution.

From this chart we see that all of the quotients determined by diluting the blood with 0.6% NaCl are lower than those determined in the original manner; *i. e.,* that in the presence of a higher chloride content less of the bromides are recovered in the blood. This meant that in a solution containing both chlorides and bromides, the former interfere with the complete recovery of the latter. To determine the extent of this influence we undertook the following experiments:

(A) In a number of specimens of blood serum we removed all the chlorides and bromides by silver nitrate and then, dissolving a known quantity of bromides in these, determined the bromide content by the Walter method, using an aqueous solution of bromides as a standard. The results were as follows:

1	Serum with 20 mg. per 100 cc.	Result 21 mg. per 100 cc.
2	24	25
3	28	27.2
4	40	41
5	48	49
6	56	58

These results show that the bromides introduced are recovered with slight variations above and below the actual amount introduced, the variations most probably depending upon the usual range of error in colorimetric determinations.

(B) Similar determinations were carried out in spinal fluid from which the chlorides and bromides were removed by silver nitrate, with the following results:

1	Spinal Fluid with 20 mg. per 100 cc.	Results: 21.5 mg. per 100 cc.
2	22	21.5
3	24	24.7
4	26	24.5
5	28	28.9
6	30	28.5
7	32	29.5
8	40	40.8
9	48	49.0
10	56	58.0

These results are similar to the ones obtained with the blood serum.

(C) Known quantities of bromides added to distilled water:

1	Water with 100 mg. per 100 cc.	Results 100 mg. per 100 cc.
2	25	25.5

The addition of NaCl to the water, however, gave different results, viz.:

1	0.2% NaCl with 100 mg. of bromide.	Results 86.5 mg. per 100 cc.
2	0.3%	78.0
3	0.6%	73.7

These results show that in chloride-free water the recovery of bromides is similar to that of chloride-free serum or spinal fluid. In the presence of chlorides, however, there is a loss of some of the bromides, the loss increasing with the increase in chloride content.

(D) A similar loss of bromides was found in experiments with known quantities of bromides dissolved in blood serum and cerebrospinal fluid from which the chlorides were not removed.

Conclusions. (1) The determination of the bromide content of a fluid containing chlorides, by the Walter method, is influenced by the amount of chlorides in that fluid. The loss of bromides is directly proportional to the amount of chlorides. Blood serum, cerebrospinal fluid, and water are, within narrow limits, similar in this respect. (2) The determination of the bromide content by the Walter method in chloride-free fluid is reliable, the margin of error being quite limited. (3) Blood and cerebrospinal fluid containing similar quantities of chlorides would be liable to the same error, and the bromide contents obtained would be reliable for purposes of comparison. (4) For absolute reliability the technique of the Walter method should be modified as follows: *a*. The blood should be diluted with a NaCl solution, the strength of which is equal to that of the blood. *b*. The chloride contents of the blood and cerebrospinal fluid should be determined, and in computing the actual amount of bromides allowances should be made for the loss due to the chlorides. The present investigation shows that the loss caused by 0.6% NaCl (which is the average NaCl concentration in blood serum) is about 27%. Further studies are being made to determine the exact losses due to NaCl concentrations ranging from 0.4% to 0.9%.

6803

A Modified Class Demonstration of Difference Between Ephedrine and Epinephrine Blood-Pressure Response After Cocaine.

HARALD G. O. HOLCK AND MUNIR KAN'AN. (Introduced by C. F. Schmidt.)

From the Department of Pharmacology, School of Medicine, American University of Beirut, Beirut, Lebanon.

The demonstration of cocaine sensitization for epinephrine and desensitization for ephedrine according to Sollmann and Hanzlik[1] calls for the following sequence of administration in a doubly vagotomized dog under barbital anesthesia: Epinephrine (0.05 mg. per kg.), ephedrine (2.5 mg. per kg., both intravenously), cocaine (10 mg. per kg., hypodermically), then repetition of the epinephrine and ephedrine injections after suitable intervals of time.

The epinephrine dosage should be 10 times less[1] and even as low as 0.002 mg. is very satisfactory in atropinized dogs under amytal anesthesia.

In regard to the ephedrine desensitization by cocaine we ran 3 experiments, each with a proper control without cocaine, using the same procedure as above, but only 2 mg. of ephedrine per kg. In 2 of these pairs of experiments we could not tell any difference between the cocaine and the control experiment; the second ephedrine dose was given one hour after the first in all cases. Also, in another study involving 30 dogs with even sex distribution and graded sizes, the average second ephedrine blood-pressure rise in terms of percentage of the first ephedrine rise was 53.3 with a standard deviation of 9.5 when ephedrine was followed by ephedrine, whereas the values for another 30 dogs was 40.0, standard deviation 11.4, when we gave ephedrine, cocaine and ephedrine. Thus, although the average is 25% lower when cocaine is given, the variability in response is so great that in many cases the ephedrine desensitization to itself would give values about the same as those obtained after ephedrine and cocaine, and in some cases would give even higher values than in the control experiment.

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[1] Sollmann, T., and Hanzlik, P. J., Experimental Pharmacology, W. B. Saunders, 1928, 227, 282.

That cocaine sensitizes to epinephrine and depresses the ephedrine response may be demonstrated to a class more clearly as follows, using atropinized dogs, anesthetized with amytal.

(1) 0.02 cc. of 5% (1 mg.) cocaine per kg. injected intravenously, using 10 to 15 seconds, is shown to give but a very slight modification of the blood pressure (Fig. 1, A). Preliminary experiments have shown that this dose intravenously sensitizes to epinephrine; no attempt has been made to ascertain the minimum sensitizing intravenous dose of cocaine. We have purposely used a very small volume of cocaine solution of high concentration.

(2) 0.1 cc. of 1 33,300 epinephrine (0.003 mg.) per kg. is injected intravenously into a dog to show the normal response. A few minutes after the blood pressure has again become normal a similar dose of epinephrine is administered, and just when the initial rise has reached its maximum, 1 mg. of cocaine per kg. is injected intravenously. Any beginning fall is checked, and a moderate, somewhat prolonged rise occurs, showing the sensitization (Fig. 1, B1 and B2). The tracings show that unless the cocaine is given at just the right time (perhaps even better just before the maximum initial rise has been reached), the effect will be much less pronounced, due to the rapid destruction of the epinephrine. One may also show the full sensitization by injecting epinephrine a third time about 10 minutes later.

(3) By another tracing the typical prolonged response after intravenous injection of 0.1 cc. of 2% ephedrine (2 mg.) per kg. is demonstrated (Fig. 2, C).

Medium sized atropinized dogs, both sexes; amytal anesthesia. Time intervals, 1 minute.

COC, 0.02 cc. of 5% cocaine hydrochloride (1 mg.) per kg., intravenously.

ADR, 0.1 cc. of 1/33,300 epinephrine (adrenaline) hydrochloride (0.003 mg.) per kg., intravenously.

EPH, 0.1 cc. of 2% ephedrine hydrochloride (2 mg.) per kg., intravenously.

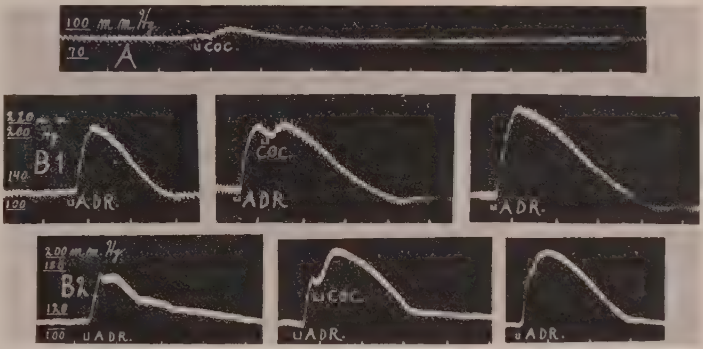

FIG. 1.

A. Effect of cocaine upon normal blood pressure.

B1 and B2. Normal epinephrine blood pressure rise. Sensitization of this response by injecting cocaine at the height of the initial rise of blood pressure. Epinephrine response after some minutes. Intervals between tracings represent about 6 minutes each.

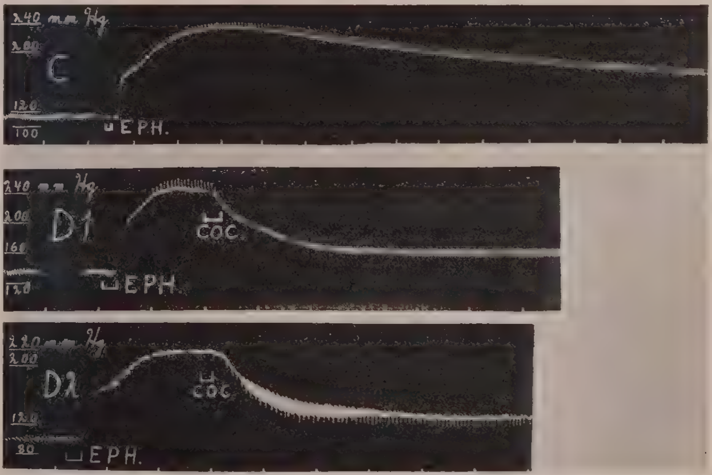

FIG. 2.

C. Effect of ephedrine upon normal blood pressure.

D1 and D2. Depression in the ephedrine response by injecting cocaine just after the maximum has been reached.

(4) Into a second dog a similar dose of ephedrine is injected intravenously. Shortly after the maximum response has been secured—2 to 3 minutes after the injection—1 mg. of cocaine per kg. is administered intravenously. There should be a sharp break in the curve, and blood pressure should reach a constant level in about 5 to 7 minutes (Fig. 2, D1 and D2). Although in these 2 tracings a constant level is reached a little above normal blood pressure, we have 2 other tracings in which it fell to normal, though not below it.

These 2 demonstrations show that upon intravenous injections of small doses of cocaine the epinephrine sensitization and the ephedrine desensitization occur almost immediately. According to Trendelenburg[2] the beneficial effect of cocaine and other local anesthetics upon the epinephrine response may in part be due to the slowing of circulation by such substances, "so that the epinephrine reaches the arterioles in concentrated solution." Our tracing B2 shows that the sensitization takes place just as well when the epinephrine is already in the arterioles.

6804

Microincineration of Tubercles.

ESMOND R. LONG.

From the Henry Phipps Institute, University of Pennsylvania.

The technic of microincineration as developed by Policard[1] and modified by Scott[2] has been used in studying the mineral content of tubercles from human tuberculosis and experimental tuberculosis in guinea pigs. Alternate serial paraffin sections were stained and ashed. The ashing process was carried out in a muffle furnace at a temperature of approximately 540°, about 4 hours being allowed for the incineration.

The general observation made by previous investigators that the nucleus is the part of the cell richest in ash, was confirmed. The distinction was found to be conspicuous in the tuberculous spleen, where the mineral-rich Malpighian corpuscles stood out sharply against a mineral-poor background, the latter being found by comparison with the stained sections to consist of aggregations of epi-

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[2] Trendelenburg, P., Die Hormone, Julius Springer, 1929, 1, 244.

[1] Policard, A., and Doubrow, S., *Ann. d'anat. path. med.-chir.*, 1924, **1**, 163; Policard, A., *Protoplasma,* 1929, **7**, 464.

[2] Scott, G. H., *Compt. rend. soc. biol.*, 1930, **190**, 1073, 1323; PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 349; Scott, G. H., and Horning, E. S., *Am. J. Path.,* 1932, **8**, 329.

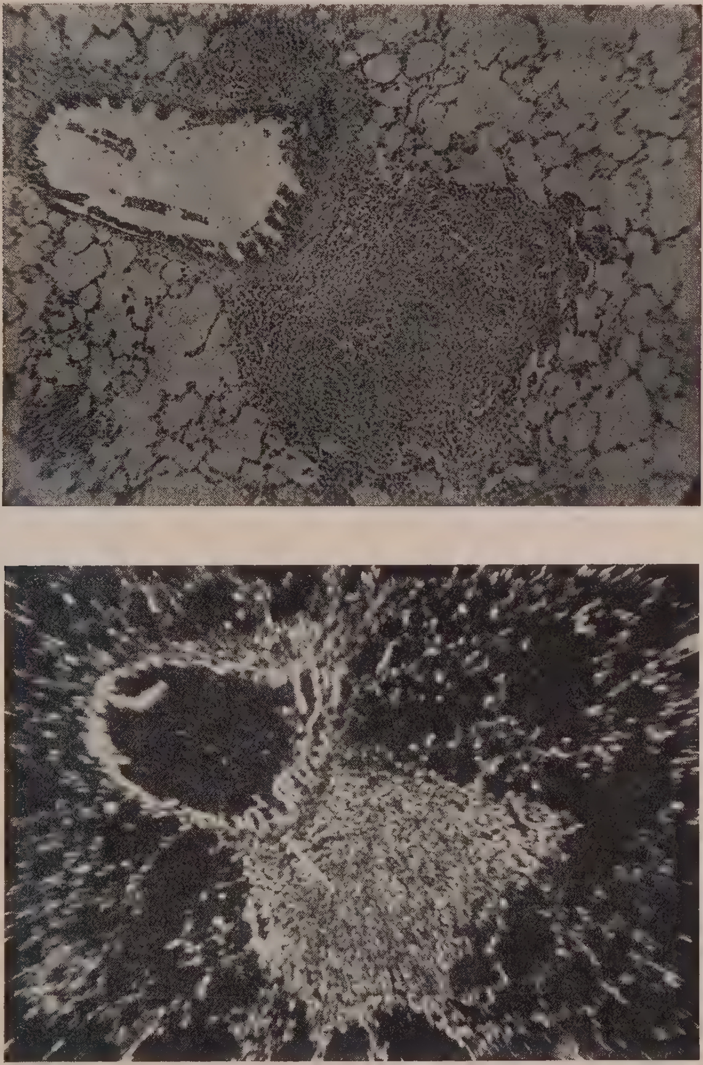

Fig. 1.

thelioid cells. In general the epithelioid cell cytoplasm was found low in ash.

As a rule regions of degeneration and recent necrosis were found to contain less ash than the corresponding undegenerated tissue. This was the case in guinea pig tubercles, acute tuberculous pneumonia in man and chronic fibrocaseous human tuberculosis. A peribronchial tubercle (guinea pig) in the corresponding stained and ashed sections is shown in the accompanying figure (Fig. 1). It will be noted that the center of the ashed tubercle contains somewhat less mineral than the outer zones, and that this region corresponds to a spot of early degeneration in the stained tubercle. It seems reasonable to attribute the loss of mineral to karyolysis and diffusion away of soluble mineral material.

In later stages of the human disease remineralization of necrotic tissue could be observed. In sections of experimental tuberculosis kindly furnished by Dr. Spies, taken from tuberculous rabbits given intensive oral treatment with viosterol, the speed of this process was found to be much enhanced (compare with other results of Spies[3]). On the other hand in 2 tuberculous guinea pigs given 50 cc. of viosterol by mouth in the course of a month in this laboratory no calcification was observed. It appears that viosterol is usually but not always effective in causing calcification in experimental tuberculosis.

6805

Reaction of Anterior Hypophysis of Immature Rat to Placental Hormones.*

J. M. WOLFE, DORIS PHELPS AND RUCKER CLEVELAND.
(Introduced by R. S. Cunningham.)

From the Departments of Anatomy and Gynecology, Vanderbilt University School of Medicine, Nashville, Tennessee.

Lehmann[1] has reported that injection of placental extracts brings about pregnancy changes in the anterior hypophysis of the female rat.

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[3] Spies, T. D., *Am. Rev. Tuberc.*, 1931, **23**, 169; Spies, T. D., and Berryhill, W. R., 1932, **26**, 275.

* This work has been supported by a grant from the National Research Council Committee for Research in Problems of Sex.

[1] Lehmann, J., *Virchow's Arch. f. path. Anat. u. Physiol.*, 1928, **268**, 346.

Collip and his associates[2] have recently reported that long term injection of the anterior-pituitary-like hormone of the placenta brings about an enlargement of the anterior hypophysis, which is later followed by a decrease.

We have carried out a series of experiments in which we have studied the histological changes in the anterior pituitaries of immature rats which had received injections of a relatively crude oestrin-free extract of placenta. The method of preparation of this extract has been described.[3] We have used litters of immature female rats, 25 to 35 days old. Half of the animals in each litter received 2 subcutaneous injections daily for 6 to 8 days. The remaining animals in the litter served as controls.

The results of the injections were constant and definite. Table I illustrates some of our findings and shows that the ovaries of the injected animals were markedly increased in size.† The swollen and hyperemic pituitaries of the injected animals exhibited a moderate but invariable weight increase when compared with those of the controls. The ovaries and pituitaries of all animals were weighed on a torsion balance accurate to 0.5 mg. (Hartmann and Braun). Analysis of Table I reveals that the increase in the size of the hypophysis was as a rule greatest in the animals in which the ovarian reaction was greatest, a fact already noted by Collip and associates.

Complete serial sections of the hypophyses of both the control and experimental animals were cut. One section from each ribbon of 25 was mounted, giving a complete set of sections. Every fifth or sixth slide of each set of sections was studied. Cell counts were made of each section studied. In this way we were able to calculate the relative percentage of each cell type present. The anterior lobes of the injected animals exhibited marked histological changes, when compared with the controls. Analysis of Table I reveals that injection of placental extract brings about a slight but constant decrease in the relative percentage of the cells of Type I (eosinophiles). However, the most marked changes were in the basophilic elements (Type III). In the anterior lobes of the controls, a majority of the basophiles were small and completely filled with violet or

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[2] Collip, J. B., Selye, H., Thomson, D. L., and Williamson, J. E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 590.

[3] Klingler, H., Burch, J. C., and Cunningham, R. S., *Surg., Gyn. and Obstet.*, 1933, **56**, 137.

† A similar hypertrophy has been obtained by the injection of pregnancy urine extracts (Follutein and Antuitran "S"). These extracts were kindly furnished by E. R. Squibb & Sons and Parke, Davis & Co., respectively.

TABLE I.
Changes Induced by Injections of Placental Extracts.

Animal No.	Type	Inj. period, days	Rat wt., gm.	Ovary wt., mg.	Hyp. wt., mg.	% of Cell Types				Sections counted	Cells counted
						Type I	Type III		Type IV		
							Gran.	Non-gran.			
228	Cont.	—	75	27	3.0	37.0	10.0	—	53.0	5	2,828
227	Exp.	6	72	83	3.5	34.2	3.6	4.5	57.7	3	918
231	Cont.	—	83	31	3.5	40.9	10.6	0.2	48.3	5	2,164
232	”	—	89	27	3.0	35.8	7.0	—	57.2	5	1,504
229	Exp.	4	74	91	4.0	32.5	4.1	5.9	57.5	5	1,733
230	”	4	77	87	4.0	31.4	4.1	6.0	58.5	5	1,849
233	”	6	72	100	4.5	32.3	6.9	5.6	55.2	5	2,043
307	Cont.	—	69	23	3.0	32.4	6.5	—	61.1	4	1,177
308	”	—	73	27	3.0	29.8	6.8	—	63.4	4	973
309	Exp.	6	67	83	4.0	27.5	2.5	5.5	64.4	5	777
310	”	6	68	77	4.5	25.0	3.0	6.0	66.0	4	901
311	” (Cast.)	6	69	—	3.5	33.6	10.3	—	56.1	4	1,200
312	” (Cast.)	6	70	—	3.5	33.0	10.0	—	57.0	4	1,015
351	Cont.	—	73	23	2.5	42.2	4.5	0.5	52.8	5	2,174
352	Exp.	6	70	79	3.5	30.6	2.9	6.6	59.9	4	1,530
353	Cont. (Cast.)	—	73	—	3.0	38.9	12.5	—	48.6	4	1,770
354	Exp. (Cast.)	6	70	—	3.0	36.1	11.5	0.7	51.7	4	1,972
‡		Total of sections studied and cells counted								177	65,432

dark blue granules. In the injected animals, these cells were larger but a great majority of them had lost all or a greater part of their granular material (Table I). This was a constant finding in all the injected animals. It was found that castration of the injected animals 2 to 3 days before the beginning of the injection period prevented this loss of granular material from the basophilic elements.

6806

Presence and Significance of Choline in Cortico-Adrenal Extract.

EDWARD EAGLE. (Introduced by J. Howard Brown.)

From the Department of Pathology, The Johns Hopkins University.

The widespread occurrence of choline and its compounds in the normal organism has been regarded as an indication that it may have some functional significance. Choline is normally found in the brain, blood, bile, intestine and adrenal cortex; as well as in extracts of the spleen, lymph nodes, thymus, pancreas, etc. In spite of the many studies that have been made, the rôle of choline in health and disease is still a matter of speculation. The antagonistic action between choline and epinephrine is of particular interest because of the normal presence in the adrenal cortex of the former, a powerful parasympathetic stimulant, and the elaboration by the adrenal medulla of the latter, a sympathetic stimulant of the highest order. The humoral possibilities of choline and acetylcholine have been widely advanced, the former as the hormone of intestinal movement by Magnus and LeHeux, and the latter as "Vagusstoff", a possible circulatory hormone, by Loewi and others. It has been reported that choline is converted into acetylcholine by virtue of the ability of the body to supply the acetyl group.[1]

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‡ Editor's Note: Part of table is here published to meet needs of condensation.

We have analyzed various cortico-adrenal extracts made by the method of Swingle and Pfiffner and have found them all to contain considerable quantities of choline. Four different tests were used on each sample of extract: 2 microscopic tests, a precipitation test, and a color test. We have studied Eschatin (Parke, Davis & Co.) and several different extracts made by Harrop *et al.** In each case, these extracts were found to give strongly positive choline results. Further quantitative work in this connection is in progress.

The question arises, is choline the hormone of the adrenal cortex or is it present in cortico-adrenal extract as a potent, disregarded impurity? To eliminate the former possibility 40 young rats were used in 4 series of experiments, and an attempt was made to maintain life with choline compounds following adrenalectomy. The untreated adrenalectomized controls all died within 3 days. Although some of the choline-treated rats survived somewhat longer than the controls, the results indicated that choline cannot maintain life in adrenalectomized rats and hence must be present in extracts of the adrenal cortex as an impurity which must be removed. Recent investigators attempted unsuccessfully to remove completely the epinephrine from their extracts. No attention has been directed to the presence of choline and its compounds. Acetylcholine has been reported to be hundreds of times more active in causing a fall in blood pressure than is epinephrine in causing a rise in blood pressure.[2] This antagonism between the pressor effect of epinephrine and the depressor effect of acetylcholine renders the application of the commonly used method for assaying the epinephrine content of cortico-adrenal extract open to question. Thus Hunt and Taveau[2] have shown that a mixture of 1 cc. of 1:2,500,000 acetylcholine plus 1 cc. of a 1:200,000 solution of epinephrine manifested an active depressor effect instead of the pressor effect anticipated from this high concentration of epinephrine. Hunt also found that acetylcholine, when injected intravenously in the cat in such extraordinarily small doses as 0.000,000,002, 4 mg. per kg., brought about a notable decrease in the blood pressure.[3] The presence of so potent an impurity in cortico-adrenal extract should not be disregarded.

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[1] Numerous references may be found in the papers of Dale, H. H., *Lancet*, 1929, **1**, 1285; and Riesser, O., *Arch. f. Exp. Path. u. Pharmakol.*, 1931, **161**, 34.

* I wish to thank Drs. Arthur Grollman and G. A. Harrop for generous supplies of the various samples of extract used in the present study.

A review of the recent literature indicates that many of the properties and results attributed to the hormone of the adrenal cortex simulate typical choline action and may very well be the result of injection of choline-containing extracts. Choline, a profound stimulant of the parasympathetic nervous system, has been reported to influence markedly carbohydrate metabolism, cause glycogen mobilization, alter the blood picture particularly with respect to the quantitative blood cellular arrangement, augment water excretion, increase respiratory metabolism, and bring about many other results—all of which have been recently attributed to the hormone of the adrenal cortex by various investigators.

We have also analyzed extracts made by the method of Grollman and Firor* and have been unable to detect choline. No epinephrine could be found in these extracts by chemical or biological methods, indicating that epinephrine is present only in traces, if at all. The relative absence of noxious by-products in the extracts made by this latter method as compared to those made by other current methods has also been emphasized by these authors.[4]

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[2] Hunt, R., and Taveau, R. de M., Bull. 73, Hyg. Lab., U. S. Public Health and Mar. Hosp. Service, Wash., 1911.

[3] Hunt, R., *Am. J. Physiol.*, 1907, **45**, 197.

[4] Grollman, A., and Firor, W. M., *J. Biol. Chem.*, 1933, **100**, 429.

6807

Preparation of an Active Undenatured Antigen from Hemophilus Pertussis.*

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Any study of the use of vaccines in whooping cough must take into consideration two basic facts, first that the causal agent is generally conceded to be the Bordet-Gengou bacillus,[1] and second, that this organism is capable of yielding high antibody titres in the vaccine treated host, as well as in the convalescent patient.[2] On purely *a priori* grounds then, one would rather expect to find formidable statistics evidencing the protective value and perhaps the therapeutic efficiency of pertussis vaccine. Yet the past 25 years has witnessed no convincing trend in this direction. That the outlook is not altogether dismal is indicated by such clinical series as those of Sauer,[3] and Madsen,[4] and it seems likely that much of the discordance in the literature reflects the multiplicity of methods used in preparation of the vaccine rather than a failure of the basic tenets of vaccine therapy.

Krueger[5] has recently emphasized the rôle of denaturation reactions in altering the antigenicity of bacterial preparations and has pointed out that these reactions probably occur to a very considerable degree during the physical and chemical manipulations incident to the killing of bacterial suspensions, as ordinarily carried out. To obviate the denaturation factor somewhat he has physically disrupted living bacterial cells of various kinds and has put into solution or suspension the cellular components released by this method. After removal of residual live cells by ultrafiltration the filtrate is employed as an antigen. The present paper details the procedure followed in making *Hemophilus pertussis* antigen of this type for clinical experimental studies in immunization and therapy.

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* The authors wish to acknowledge financial support extended for this work by the Department of Pediatrics, University of California, and a Research Grant from Eli Lilly and Company.

[1] Gardner and Leslie, *Lancet*, 1932, **1**, 9.

[2] Chievitz and Meyer, *Ann. de l'Inst. Past.*, 1916, **30**, 503.

[3] Sauer, L., *J. Am. Med. Assn.*, 1933, **100**, 239.

[4] Madsen, T., *Deut. Med. Wchnschr.*, 1929, **55**, 559.

[5] Krueger, A. P., *J. Inf. Dis.*, in press.

We were convinced by preliminary work that the present antigen should be prepared from smooth pathogenic types (Phase I of Leslie and Gardner)[6] and to that end constantly renew our stock strains, replacing the latter at brief intervals with new strains obtained from cough plates. The organisms are grown in Roux flasks on potato-extract-glycerine-agar medium enriched with 25% of human blood. Human blood is employed instead of horse blood because we desire to rule out the possibility of sensitizing our patients to foreign serum proteins adsorbed on the bacteria, and because human blood appears to maintain the active antigenic phase of *Hemophilus pertussis* more consistently.

After 48 hours' incubation at 37°C. the growth is harvested in sterile Locke's solution (pH 7.0). The suspension is given one preliminary washing in Locke's solution and is then passed through filter paper in a Gooch filter to eliminate agar particles. The bacteria are then alternately centrifuged and resuspended in fresh Locke's solution for 3 successive washings, discarding the supernatant each time. The last supernatant is always clear, and from the sediment a final suspension is made up so that each ml. contains about $5x10^{10}$ bacteria as determined by the centrifuged sediment method.[7] The heavy bacterial suspension is then transferred to a special type of ball mill[8] where it undergoes grinding for 12 hours. The ground-up suspension is filtered through a 4.5% acetic collodion membrane,[9] this final step yielding a water-clear filtrate which constitutes the antigen. To it may be added 1:1,000,000 merthiolate as a bacteriostatic without causing detectable denaturation.

The antigen regularly contains approximately 4 mg. of nitrogen per 100 ml. of which 90% is protein nitrogen. The preparation gives a positive Biuret test, a positive Molish test, a positive xanthroproteic test, a negative Hopkins-Cole reaction, a positive Adamkiewicz test, and a negative Millon test.

It is distinctly non-toxic, as evidenced by our failure to elicit temporary or cumulate toxic effects in rabbits and guinea pigs by daily subcutaneous, intradermal and intravenous injections. Intradermal injections of antigen into rabbits followed within 20 hours by intravenous injection of the same material failed to produce local tissue reactions such as those reported by Mishulow,

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[6] Leslie and Gardner, *J. Hyg.*, 1931, **31**, 423.

[7] Krueger, A. P., *J. Gen. Physiol.*, 1930, **13**, 553.

[8] Krueger, A. P., *J. Inf. Dis.*, in press.

[9] Krueger, A. P., and Ritter, R. C., *J. Gen. Physiol.*, 1930, **13**, 409.

Mowry and Scott[10] who used toxic filtrates of *H. pertussis*. If, however, the final intravenous injection was performed with a live suspension of the organism after preliminary skin sensitization with the present antigen, a typical local erythematous response, later progressing to skin necrosis, was evinced.

Rabbits immunized by combined intradermal and subcutaneous injection of the antigen were capable of withstanding intravenous injection of 2×10^{10} virulent organisms—an amount which kills control animals of like weight within 20 hours. It did not seem likely that further protection experiments with animals not naturally susceptible to clinical whooping cough would yield information of any value. Therefore, after adequate experiments on the clinical and laboratory staff to demonstrate its harmlessness to humans, the antigen was subjected to clinical trial.

6808

Effect of Desiccated Beef Testis Upon Sex Glands of the Rat.

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From the Departments of Pharmacology and Pathology, University of Oregon Medical School.

The prostate gland of the rat shows a marked increase in weight in aged males.[1] McGee[2] and Gallagher and Koch[3] have shown that the testicular hormone of the beef testis is lipoid soluble. Moore and McGee[4] state that the lipoid fraction of the beef testis possesses the same activity as the internal secretion of the testis, judged by its effect on spermatozoa in isolated epididymes.

We have fed beef testis to mature male rats in an attempt to determine whether it might have any effect on the increase in weight of the prostate gland, as it tends to occur in the aging animals. The testes were ground and desiccated at 65°C., without removal of fat or extractives. The desiccated testis was then refrigerated and fed in 0.2 gm. amounts, 5 times a week.

The experimental animals gave no visible evidence of departure from the condition of litter-mate controls. When sacrificed however, the testes, prostate gland and seminal vesicles were found to be considerably smaller than in the control animals. There was no contrast in the weights or gross appearance of the livers and spleens in the 2 groups. Table I presents the weights of the control rats together with the weights of their testes with epididymes, prostate glands and seminal vesicles. The average weights of these organs are given and the fractional proportion of the average total body weight represented by each organ is stated. Table II presents the same figures for the experimental animals, together with the number of doses of desiccated testis received by each and the period

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[10] Mishulow, Mowry and Scott, *J. Immunol.*, 1930, **19**, 227.

[1] Donaldson, H. H., The Rat. Wistar Institute, Philadelphia. 1924.

[2] McGee, L. C., *Proc. Inst. Med. Chicago*, 1927, **6**, 242.

[3] Gallagher, T. F., and Koch, F. C., *J. Biol. Chem.*, 1929, **84**, 495.

[4] Moore, C. R., and McGee, L. C., *Am. J. Phys.*, 1928, **87**, 436.

TABLE I.
Weight of Sex Glands of Control Rats.

Rat No.	Total Wt.	Testes and Epididymes	Prostate	Seminal Vesicles
	gm.	gm.	gm.	gm.
20	320	5.47	.49	.63
21	300	4.26	.46	.34
22	325	4.41	.38	.51
23	327	4.55	.66	.82
24	271	4.30	.54	.30
25	264	4.80	.41	.43
26	351	5.85	.61	.80
27	315	4.30	.84	.53
28	280	4.10	.75	.35
29	295	4.30	.75	.50
41	254	4.59	.33	.23
43	300	4.46	.20	.17
Average:	300	4.61	.535	.467
Fractional wt.:		0.015%	.0017%	.0015%

TABLE II.
Weight of the Sex Glands of Experimental Rats.

Rat No.	Total Wt.	Testes and Epididymes	Prostate	Seminal Vesicles	Doses (0.2 gm.)	Days*
	gm.	gm.	gm.	gm.		
6	236	2.1	.15	.06	74	85
7	275	3.5	.33	.06	74	85
35	240	1.23	.095	.07	55	99
38	240	1.15	.10	—	55	99
39	245	0.30	.085	.05	68	114
40	245	1.55	.118	.04	70	117
46	340	1.68	.265	.10	55	99
Average:	260	1.64	.162	.063		
Fractional wt.:		0.0063%	.00062%	.00024%		

* Days intervening between first and last dose of desiccated testis.

over which the feeding extended. It will be noticed that the weight of the sex glands is considerably less in the experimental animals than in the controls. The difference in weight of the sex glands of the 2 groups is well shown on comparison of the proportionate weights of the glands to total body weights.

The atrophic appearance of the sex glands was most striking in those animals fed the desiccated testis over the longest periods of time. Apparently the duration of the experimental feeding of testis is of more importance than the equivalent dose given in a shorter period of time. Further experiments are in progress to verify this opinion.

An analysis of the histologic findings in the sex glands of the control and experimental rats is given by W. C. Hunter, as follows: "Histologically the prostate glands and seminal vesicles of all experimental animals display evidences of parenchymal reduction. The process has all of the characteristics of simple atrophy as shown by pronounced shrinkage in the size of individual glandular units, leading not infrequently to complete collapse of alveoli with a disappearance of the majority of cells and high grade atrophy of those still remaining. The epithelial cells are small, cubical rather than columnar in form and exhibit varying degrees of nuclear pyknosis. The stromal portion of both prostate and seminal vesicle is in every instance unchanged, indicating clearly that the reduction in size has been accomplished by atrophy and shrinkage of the glandular element. The changes described are much more pronounced in the rats fed the testicular substance over the longer periods of time."

"Similarly there seems to be a correlation between the duration of the experiments as regards the testicular changes in the various rats. The testicles of rats fed for a comparatively short time display no abnormalities on the part of either interstitial cells or those concerned with spermatogenesis. The animals fed testis for a longer period of time show aspermia and clear-cut evidence of degeneration on the part of certain cells, notably the spermatids and primary spermatocytes, thus accounting for the small size and condensation of seminiferous tubuli. The interstitial cells of Leydig show an increased density of nuclear chromatin and of lipochrome substance in the cytoplasm, but no quantitative decrease of cells."

Cryptorchidism with resultant atrophy of the testis does not figure as a possible explanation of the changes found in the sex glands since the testes were noted to be in the scrotum of each experimental animal at the time of beginning of the experiment and up to the time of its completion. Vitamin deficiency with the possible deleterious effects upon the testes[5] was avoided through providing a food mixture known to be adequate and adding codliver oil, yeast, fresh greens and fresh meat.

6809

Chronaxie in Morphine Addicted Rats on High and Low Calcium Diets.

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Experiments by one of us (L. E. D.) have shown that the changes in water content of tissues and blood, and the characteristic symptoms accompanying morphine addiction and withdrawal in dogs and rats can be ameliorated by the use of a high calcium diet together with injections of "parathormone". This suggests a parallel with parathyroid tetany. In this latter condition there is little change in the chronaxie of motor nerves, but a decided increase in the chronaxie of skeletal muscles; this high chronaxie is, however, reduced to the normal by administration of "parathormone."[1]

To determine objectively, if possible, the difference in excitability of our rats on the calcium treatment and those not on the treatment, chronaximetric determinations were made. The composition of the diets was as follows:

	Diet No. 1	Diet No. 2
Whole Wheat	60%	63%
Casein	15	15
Whole Milk Powder	10	10
Alfalfa Meal	5	5
Butter	0	5
Calcium Lactate	5	0
Cod Liver Oil (fortified with ergosterol)	5	0
Sodium Chloride	0	2

The rats were first addicted by daily injections of morphine sulphate in doses increasing from 20 mg. to 50 mg. per kilo body weight over an interval of 2 weeks. At the chosen time each rat was decerebrated under ether anaesthesia, one sciatic nerve being used for peripheral stimulation, the other for central stimulation, the chronaxie being determined every 15 minutes during the second hour after decerebration when the readings had become constant. Three rats were used as controls on each diet, and 3 morphinized rats for chronaxie measurements on the last day of addiction, and 3 for each of the 3 days following withdrawal. In the tables each result is the average of 3 individuals. Thus in Table I the value .11 for peripheral stimulation in the control group

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[5] Evans, H. M., and Bishop, K. S., *Am. J. Phys.*, 1922, **63**, 396.

[1] Parhon, C.-I., and Kreindler, A., *C. R. Soc. Biol.*, Paris, 1931, **107**, 398.

TABLE I.
Diet No. 1, "Calcium-treated Group."

Time after decerebration		Controls		Last Day of Addiction		1st day of Withdrawal Average of 3		2nd Day of Withdrawal		3rd Day of Withdrawal	
		R	C	R	C	R	C	R	C	R	C
1 hr.	Peripheral	.52	.11	.66	.14	.84	.09	.50	.09	.31	.14
	Central	2.46	.12	1.60	.10	2.40	.08	1.50	.09	2.13	.10
1:15	Peripheral	.53	.09	.89	.10	.94	.09	.53	.07	.34	.12
	Central	2.65	.10	1.90	.10	2.60	.08	1.57	.09	2.25	.10
1:30	Peripheral	.53	.09	.95	.10	.94	.09	.57	.07	.35	.05
	Central	2.70	.09	2.10	.09	2.90	.07	1.63	.08	2.60	.08
1:45	Peripheral	.59	.09	1.30	.09	1.21	.09	.62	.07	.41	.08
	Central	3.00	.08	2.44	.09	3.60	.07	1.60	.08	2.60	.08
2:00	Peripheral	.64	.09	1.45	.09	1.81	.08	.62	.07	.51	.08
	Central	2.10	.08	2.59	.08	3.80	.06	1.67	.08	3.11	.08

R = Rheobase in volts.
C = Chronaxie in thousandths of a second.

TABLE II.
Diet No. 2, "Untreated Group."

Time after decerebration		Controls		Last Day of Addiction		1st day of Withdrawal Average of 3		2nd Day of Withdrawal		3rd Day of Withdrawal	
		R	C	R	C	R	C	R	C	R	C
1 hr.	Peripheral	.56	.10	.34	.16	.36	.12	.26	.16	.26	.14
	Central	1.22	.11	1.03	.21	.56	.07	1.50	.10	1.10	.09
1:15	Peripheral	.64	.09	.48	.14	.46	.12	.35	.10	.26	.11
	Central	1.15	.12	1.18	.14	.55	.07	1.53	.07	1.33	.08
1:30	Peripheral	.72	.09	.43	.13	.58	.12	.43	.10	.33	.10
	Central	1.15	.09	1.41	.12	.58	.06	1.76	.09	1.60	.07
1:45	Peripheral	1.00	.09	.42	.12	.75	.13	.45	.08	.37	.08
	Central	1.38	.10	1.58	.11	.56	.07	2.00	.06	1.79	.07
2:00	Peripheral	1.65	.09	.50	.11	.76	.12	.50	.08	.41	.08
	Central	2.20	.10	1.58	.11	.59	.07	2.20	.06	1.91	.07

R = Rheobase in volts.
C = Chronaxie in thousandths of a second.

one hour after decerebration is the average of .11, .09, .14; the chronaxie of .12 for central stimulation is the average of .12, .12, .14; 2 hours after decerebration the value .09 is the average of .08, .08, .10 for peripheral stimulation and .08 is the average of .09, .06, .08 for central stimulation.

The chronaxie of control rats, i. e., non-morphinized, on high calcium diet and "parathormone" differed but little from those on low calcium diet. This is in contrast to the immediate effects of intravenous injection of a soluble calcium salt into the decerebrate cat, where the rheobase is raised and the chronaxie lowered.[2] On the last day of addiction both the "central" and "peripheral" chronaxies of rats on the high calcium diet and "parathormone" were slightly, but not significantly, lower than those of rats on the low calcium diet. During the 3 day period of withdrawal from morphine, the chronaxies were, if anything, slightly lower in practically every case, the differences again being hardly significant.

It may be said, therefore, that neither in normal rats nor in those subjected to morphine addiction and withdrawal does a diet rich in calcium and injection of "parathormone" significantly change the chronaxie of a typical motor nerve or of the flexion reflex.

6810

Standardization and Relative Purification Technique with Plant Virus Preparations.*

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The usual procedure in the preparation of an infective juice for inoculation or other experimental purposes, in the case of a virus like that of typical tobacco mosaic, consists first in thoroughly crushing or grinding the fresh tissue and then separating the coarser material by filtering off the clearer juice through cotton or cloth. Pressure also may be applied to the crude pulp. The crude juice thus prepared will contain the virus in what appears to be the original concentration. Often this procedure may be improved upon by a simple freezing and pressure technique. With the latter the softer tissues (young stems and leaves) are frozen for a few hours at —20°C. or below, for a period of 4 hours or less, then pressure of 10,000 lbs. or more per square inch is applied. With this treatment a clearer juice is obtainable.

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[2] Woden, L.-J., *C. R. Soc. Biol.*, Paris, 1931, **106**, 462.

* This work was made possible, in part, through a grant from the Research Committee, Graduate School, University of Wisconsin.

By the method of grinding commonly employed, the crude juice contains varying quantities of starch, proteins and other solid and highly viscous reserve products; likewise chlorophyll, plastid, and protoplasmic particles. If ground in a mortar there is also a large fraction of comminuted cell wall material. The presence of these coarser suspension and colloidal particles is unfortunate in such studies as involve, for example, filtration, adsorption and aggregation, cataphoresis, or various other physical or chemical treatments. Moreover, these coarse particles may vary widely in quantity, and will be related to significant changes in viscosity with plants grown under different environmental conditions. They also interfere with quantitative work involving accurate standardization.

The elimination of coarser particles has been variously accomplished, filtration and centrifugation being often employed. The ordinary laboratory centrifuge alone is inadequate. Filtration through porous filter candles or even alundum cups is slow and likely to give a product very diverse in virus concentration, depending upon kind and quantity of viscous materials present.

In the development of a tentative standard method I have had in mind not a purified virus,[1] but rather a virus suspension reasonably free of coarser materials and still in a reasonably "natural" environment of other solutes. Various clarification processes have been tried. The method finally employed involves removal by precipitation and perhaps selective adsorption of the major part of the contaminating particles by means of diatomaceous earth, leaving the virus in suspension in the clarified juice. The finer grades of commercial diatomaceous earth (Kieselguhr) may be employed, but I have used for ordinary work a special product known as supercelite. Diatomaceous earth is widely used in various filtration processes.

A simple, standard procedure is as follows: The crude juice from diseased plants, (infectious juice, designated hereafter I.J) is obtained by grinding finely the leaves and young stems in a food mill and then expressing the juice through a double thickness of cheese cloth. This full-strength juice (which may be written 1/1) may be diluted, for best results, with 9 parts of distilled water (= 1/10 I.J.); to this is added with stirring 10 gm. of "celite" per 100 cc. of the diluted I.J. This is allowed to stand 30 minutes, shaking frequently, the preparation is then centrifuged at about 4000 r.p.m. for 4-5 minutes. The resulting supernatant liquid is, or should be, clear and very slightly flavous in color. All material so treated will be briefly referred to as C.T.I.J. (celite treated infectious juice). If the natural (1/1) I.J. is employed, the removal of larger particles and pigment is not so complete; with 1/5 I.J. the purity is intermediate, but obviously this last may be a desirable concentration for the treatment when it is desired to inoculate with 1/10 I.J., and when subsequent treatment of the virus may necessitate further dilution with a liquid.

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[1] Various studies have been carried out by other investigators looking toward the purification and standardization of plant viruses, among which are the following: Brewer, P. H., Kraybill, H. R., Samson, R. W., and Gardner, M. W., *Phytopath.*, 1930, **20**, 943; McKinney, H. H., *J. Agric. Res.*, 1927, **35**, 13; Vinson, C. G., *Phytopath.*, 1932, **22**, 965; Vinson, C. G., and Petre, A. W., *Bot. Gaz.*, 1929, **87**, 14; Vinson, C. G., and Petre, A. W., *Contrib. Boyce Thompson Inst.*, 1931, **3**, 131.

The 1/10 C.T.I.J. may be prepared as clear and free from color as many of the more elaborately "purified" virus preparations, if the operator selects the top parts only (ca. 10 cm. of the leafy shoot) from diseased plants growing rapidly under favorable conditions. Color of the sample then seems to be related especially to yellowing of the plants, low humidity, and other unsatisfactory conditions.

All satisfactory methods of establishing a known titer of "artificially" communicable plant viruses must consider, (1) dilution, and (2) technique of inoculation. While water (especially distilled water) is commonly used in dilution, it is not certain that this procedure is best. This paper will not deal quantitatively with this point, but an adequate dilution is necessary to arrive at a satisfactory estimate of influences and reagents upon the virus. With the needle-prick and scratch technique of inoculation here used a dilution of 1/1000 is about the highest with which a disease percentage of 90-100 may be rather consistently obtained. Other factors, however, influence the disease percentage.

This proximate purification by means of diatomaceous earth adds nothing undesirable to the virus suspension, does not noticeably shift the pH, and yields a product which may be further used in purification work. Greater dilutions (1/100, 1/1000, etc.) may be prepared in the usual way, with distilled water, or with juice prepared from healthy plants.

If it is very desirable to decolorize the virus suspension still further, also undoubtedly removing additional proteins, this may be accomplished by filtering through a thin stratum of charcoal on an asbestos mat in a Gooch crucible, or shaking up momentarily with certain grades of charcoal.

Various charcoals differ widely in the capacity to "clarify" any particular liquid. Norite, Nuchar 2, XXX, W, 00, give rather rapid decolorization of the tobacco virus suspension, but Norite and Nuchar XXX especially are among the charcoals which strongly, and more or less irreversibly, adsorb or inactivate the tobacco virus from tobacco, so that the use of various charcoals is to be recommended only in special cases when decolorization is important, and when also adequate controls may be provided. It seems probable that a special study would need to be made of the relation of any charcoal to the particular virus juice complex.

The influence of the coarser and more viscous particles of the crude juice on filtration of the virus requires special consideration. Comparing a sample of 1/10 C.T.I.J. with the same concentration of natural I.J., the former, the treated juice, had the following advantages: (a) rapidity of filtration, (b) filtration at lower pressure, (c) relative constancy of virus concentration in the filtrate, and (d) higher virus concentration of the filtrate. For example, in one experiment in which a 12 cm. Mandler filter candle was used with the usual mantle and agar set-up, the filter flask being connected with a water pump aspirator, the celite-treated I.J. filtered readily and the air pressure was reduced to about half an atmosphere by the time the desired amount of filtrate, 15 cc., was obtained. With the natural 1/10 I.J. the pressure was reduced to the full capacity of the apparatus, about one-fifteenth of an atmosphere, and a longer time interval was required to obtain 15 cc. These two samples were tested for efficiency in disease production, with results as shown in Table 1.

It is clear that the viscous materials in the natural juice markedly impeded the passage of the virus particles across the walls of the filter. The infectiousness of the filtered celite-treated juice is not far below, and usually more nearly the same as, the unfiltered controls (averaging 90-100% diseased at a dilution of 1/1000).

The results of numerous experiments have shown clearly that the celite treatment has no marked influence, either immediate or delayed, on the activity of the virus of typical tobacco mosaic. (Table 2).

TABLE I.
Infectiousness of virus of typical tobacco mosaic—natural juice contrasted with celite-treated, both filtered through a Mandler filter candle at 1/10, then diluted to 1/100 and 1/1000 for inoculation.

Character of I.F.	Dilution for inoc'n	Plants inoc'l'd	Plants dis'd	% dis'd
Natural, filtered	1/100	10	5	50
" "	1/1000	10	0	0
Celite-treated, filtered	1/100	10	9	90
" " "	1/1000	10	7	70
Control, C. T., not filtered	1/100	10	10	100

TABLE II.
Effect of time on infectivity of natural and Celite-treated infectious juice. Samples held at 1/10 during the various intervals at 6-8°C., 10 plants inoculated at each dilution, after each time-period, with each sample.

Natural I.J. Dilution for inocl'n	hrs.	Diseased %	Celite-treated I.J. Dilution for inocl'n	hrs.	Diseased %
1/10	0	100	1/10	0	100
1/1000	0	90	1/1000	0	80
1/10	24	100	1/10	24	100
1/1000	24	100	1/1000	24	100
1/10	66	90	1/10	66	80
1/1000	66	90	1/1000	66	80
1/10	240	95	1/10	240	100
1/1000	240	85	1/1000	240	85

TABLE III.
Infectiousness of virus of yellow mosaic of tobacco. C.T. juice contrasted with natural juice. Virus treated at 1/1 and 1/10 dilution; 10 plants inoculated in each experiment.

Type of treatment	Dilution for inoculation	Virus treated full strength, 1/1		Virus treated at 1/10	
		Inoc'l'd immed.	Inoc'l'd after 48 hr.	Inoc'l'd immed.	Inoc'l'd after 48 hr.
Celite-treated juice	1/100	100	100	100	90
	1/1000	90	100	100	80
	1/10,000	20	20	30	50
	1/50,000	0	20	10	10
Natural juice	1/100	As control, untreated, stock virus at 1/10 dilution		100	100
	1/10,000			20	30

For comparison are given the results of one experiment with the Celite-treatment of yellow mosaic of tobacco (Table 3). Inoculations were made (a) immediately after treatment and again (b) after 48 hours, the treated and untreated juices being held for the interval mentioned in corked vials at laboratory temperature. The irregularities recorded at the higher dilutions are not infrequent, especially after the virus has been standing.

For the present the proximate purification here proposed has found wide use in our work. This in no way minimizes the pressing importance of a highly purified virus product, if obtainable, with its attending biochemical possibilities; yet it is always possible that purification beyond a certain point may lead to inactivation.

6811

A Modification in the Thermostromuhr Method of Measuring Flow of Blood.

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The proper construction of the unit applied to the blood vessel is fundamental to the success of the thermostromuhr method of measuring flow of blood. Rein[1] developed the thermostromuhr and named the unit the diathermy thermo-element. This diathermy thermo-element is U-shaped and requires the use of collodion to hold it firmly on the blood vessel during an experiment. It is very satisfactory for short-time observations, but is less so for experiments in which the unit is to be kept on the blood vessel for several days.

Since it was obvious that a drop of collodion could not be expected to hold the unit to the blood vessel firmly enough to insure good electrical contact when the animal was allowed to run about, it was necessary to construct a unit better suited to such conditions. Consequently the unit described here was developed.

The dimensions of each particular unit depend on the size of the blood vessel to which it will be applied. Several units of varying sizes must be at one's disposal in order to permit the selection of the one which best fits the particular blood vessel. For a vessel the diameter of which is somewhat larger than 5 mm., the specifications for the transparent bakelite block are: length 19 mm., width

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[1] Rein, Hermann, *Die Thermo-Stromuhr. Ztschr. f. Biol.*, 1928, **87**, 394. In: Abderhalden, Emil, *Handbuch der biologischen Arbeitsmethoden.* Berlin, Urban and Schwargenberg, 1929, **5**, **8**, 692.

8 mm. and height 7 mm. The length of the block is determined by: (1) the distance from platinum electrode to thermojunction, which must be at least 1.2 times the diameter, that is, 6 mm. in this case; (2) platinum electrodes, which for this particular vessel are 4 by 4 mm., and (3) allowing 1.5 mm., at each end for suture holes.

The width and height are determined by the diameter of the hole into which the blood vessel is inserted (in this particular case 5 mm.), and by the thickness of wall necessary for sufficient rigidity and for countersinking the 4 leads.

After the bakelite block is cut, a hole 5 mm. in diameter is drilled lengthwise somewhat eccentrically so as to allow sufficient space on one side for countersinking the rubber tubing which insulates the lead wires (Fig. 1). Before cutting the slit at the top of the unit through which the blood vessel is slipped, the various grooves necessary for inserting the differential thermocouple circuit and the wires leading to the heating plates are made. A detailed description of the latter process may be found in a previous paper[2]; the only change in technic is the curving of the heating plates to fit the curvature of the unit before inserting them. The electrical wires may be sealed in the bakelite block with resin or bakelite cement. The latter was used with the hope that it would permit sterilization of the unit by boiling. However, the problem of sterilization of the unit has not thus far been solved satisfactorily.

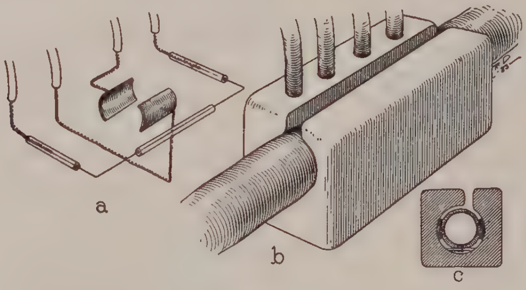

FIG. 1.

The modified diathermy thermo-element. *A* shows the wiring only. The constantin wire is represented by straight lines and the copper by coiled; *B* shows its application on a blood vessel; *C* shows a cross section of the blood vessel as it fits against the electrodes (platinum plates) and on a thermojunction.

The most satisfactory method of sterilization was accomplished by enclosing the wire leads from the unit in a piece of rubber tubing

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[2] Herrick, J. F., and Baldes, E. J., *Physics*, 1931, **1**, 407.

1 cm. in diameter. This was boiled for 15 minutes while the diathermy thermo-element was held outside of the sterilizer. Subsequently the unit was washed with water and green soap, after which it was submerged in a 1:400 solution of merthiolate for 15 minutes.

The new design does away with the use of any material for holding the unit on the blood vessel (Fig. 1). After the blood vessel is carefully isolated, it is momentarily collapsed and slipped through the slit in the top of the diathermy thermo-element. This modified diathermy thermo-element rides on the vessel, maintaining electrical contacts in spite of any movements of the animal. Holes may be drilled in the corners or sides of the unit for suturing it firmly so as to keep it from slipping along the blood vessel.

This modified diathermy thermo-element is much easier to manipulate and can be applied in considerably less time than the original unit. It has been found particularly convenient for measuring flow of blood in the thoracic portion of the inferior vena cava where movements with large amplitude occur. These units are applied, employing the usual sterile surgical technic and ether anesthesia. After the effects of the anesthesia have worn off, the flow of blood returns to normal and measurements of the flow may be carried on for several days. Undoubtedly the measurements under these conditions may be said to be more nearly normal than those made by any method thus far developed.

6812

Development of Transplanted Rat Eggs.

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The first experiments employing transplantation of mammalian eggs were reported by Heape[1] more than 40 years ago. He transferred the eggs of an Angora rabbit into the uterus of the Belgian hare. Similar work is reported by Hammond[2]. As a part of the program of work on the development of the rat, this experiment has been repeated as a test for egg viability and its capacity for continued development. The transplantations were performed between white and hooded rats in order that the pigmentation could be used as a decisive criterion of the original maternal origin of the developing eggs. Since pigmentation develops at the close of the first half of pregnancy, it is not necessary to carry the young completely through the gestation period to study the survival of the transplanted eggs.

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[1] Heape, *Proc. Roy. Soc.*, 1890, **58**.

[2] Hammond, *Verh. d. 1er Internat. Kaninschenzüchten-Kongr.*, 1930.

The animals are mated and the Fallopian tube of one side is removed from both the donor and the recipient females at the desired interval after fertilization; the eggs are secured from the Fallopian tube and the donor's eggs (hooded) are transferred to the recipient's (white) uterus. The effects of the operative procedure have been tested and the results show[3] that one horn of the uterus can be removed without disturbing the development of the embryos contained within the opposite horn. The horn of the uterus can be transferred to a subcutaneous environment[4] without interfering with the development of the subsequent litter. Further tests were made under the conditions peculiar to the present experiment and the Fallopian tube of one side was removed at the close of the third day of the gestation period. The young develop in the horn of the side of operation and were born alive in nearly 90% of the cases (13 litters out of 15) with no change in the length of the gestation period.

The experiments fall into 3 groups; (1) those in which the recipient and donor eggs were in equivalent stages; (2) those in which the transplanted eggs were of a later stage than those found in the tubes of the recipient; (3) those in which the donor eggs were of a stage earlier than those of the recipient. These 3 series of transplantations give definite differences in result.

The transplantations are performed when the donor eggs (hooded) are in the 2 and 4 cell stages, 48 hours after the formation of the copulation plug. The eggs from the Fallopian tube of the recipient are removed and the stage of their development determined. The donor (hooded) eggs are then inserted into the recipient's uterine horn by means of a pipette.

The transplanted eggs when equivalent in stage to those of the recipient implant normally in the uterus and frequently maintain their normal growth rate. In five such experiments in which 18 eggs were transplanted development proceeded normally in 60% of the embryos.

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[3] Nicholas, *Anat. Rec.*, 1925, **31**.

[4] Nicholas, *Sci.*, 1931, **74**.

When the eggs from the donor (hooded) strain are of a later stage than those of the recipient animal, only 10% developed (2 out of 20 egg transplants).

When the eggs from the hooded strain (donor) were transplanted at a stage younger than those secured from the recipient animal, implantation occurred in 80% (16 out of 20). In these cases, some of the eggs of the recipient animal had entered the uterine horn before the donor eggs (hooded) were introduced. The embryos from the 2 sources were spaced at regular intervals in the uterine horn. The development of the donor (hooded) embryos was, however, noticeably retarded when compared with those developing from the recipient (white) mother.

Development was permitted to continue to the end of the period of gestation in several cases and the young were born normally. In the cases in which the eggs of the donor and recipient were of the same stage of development, the young were all born at the same time. When the donor eggs were of later stage than those of the recipient the gestation period was not completed in any experiments so far performed. The donor eggs which were transplanted when they were in an earlier stage of development than those of the recipient were born later than the ones normal to the recipient. The maximum interval so far obtained is 48 hours, resulting in the prolongation of pregnancy to that extent as well as involving a separate second birth process 2 days later than the first.

Further experiments are in progress to determine how far this process of increasing the gestation period can be prolonged by varying the differences in the ages of the donor and recipient.

6813

Oxygen Consumption of Egg: A Quantitative Study of Oxygen Consumption of Single Developing Eggs (Orthoptera).

J. R. BURKHOLDER. (Introduced by J. H. Bodine.)

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Respiratory metabolism has been determined and correlated with the morphological development of single grasshopper eggs (*Melanoplus differentialis*) from the time of laying to hatching at constant temperature of 28° C.

A rhythmic rise and fall in the oxygen consumption occurs during the successive stages. A gradual increase takes place up to about the ninth day (Fig. 1, I) which marks the peak during the early formation and differentiation of tissue. From this point the usage of oxygen steadily falls until the embryo enters into a state of quiescence (diapause), on or about the 21st day. During diapause, the embryo uses the minimum amount of oxygen. After the diapause block is destroyed and active development begins (Fig. 1, Ia) there is an immediate rise in oxygen consumption which continues through the remaining 3 morphological periods, characterized respectively by the revolution of the embryo (Fig. 1, II), the engulfment of the yolk by the growth and closure of the lateral walls (Fig. 1, III), and the period of rapid growth ending with hatching (Fig. 1, IV).

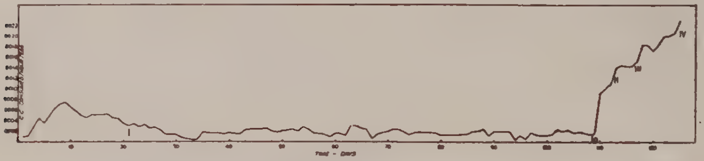

FIG. 1.

Rates of oxygen consumed during the entire development of typical egg at 28°C. Roman numerals designate the end of each developmental period; ordinates, cubic centimeters of oxygen consumed per hour per egg; abscissae, time in days indicated.

The total volume of oxygen consumed by each egg, as well as the percentage of the total volume used during each period of embryonic development, has been measured. The volume used by various eggs during corresponding intervals of growth shows a close correlation irrespective of: (a) the difference in total volume used; (b) the differences in volume used during respective periods; (c) the differences in time required to cover the respective developmental interval. When the oxygen consumption for a period varies either as to amount of energy used or time required to complete that phase of development, the following period tends to compensate for the difference.

6814

Mercapturic Acid Formation in Rabbits.

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The influence of diet on the detoxication of brombenzene in rabbits was studied by Abderhalden and Wertheimer[1], who found that an acid diet favors, while a basic diet inhibits the formation of mercapturic acid. This result was confirmed in our laboratory, using the same technique and method of determining mercapturic acid—isolation of the product by the Baumann-Schmitz method[3]. However, it was thought that the large urine volume might be responsible for the negative result obtained on the basic green-food diet, and preliminary experiments on this urine with various solvents indicated the presence of a small amount of mercapturic acid which could be extracted with chloroform.

Three rabbits were put on a diet of celery, lettuce and cabbage until the urines were definitely alkaline, and on 3 successive days each animal received 1.5 gm. brombenzene daily by subcutaneous injection. The combined urines for 6 days were divided in 2 portions, one of which was treated according to the Baumann-Schmitz procedure, and the other was extracted with chloroform. Not a trace of mercapturic acid was obtained by the former method, while the chloroform extract yielded 0.96 gm. of crystals, a yield corresponding to 3.5% of the brombenzene injected. The substance melted at 153-4°, gave a positive thiophenol reaction with concentrated sulfuric acid, and its barium salt analyzed 17.60% Ba, the calculated value for mercapturic acid being 17.81%. The experiment was repeated on another rabbit with a similar result—no mercapturic acid by the Baumann method, 2.7% yield by the chloroform extraction method.

When applied to the urine of a dog that had received 9.0 gm. of brombenzene over a period of 6 days, the chloroform extraction method proved to be more efficient than the Baumann-Schmitz and the Baumann-Preusse[2] methods, as shown by the following results:

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[1] Abderhalden, E., and Wertheimer, E., *Arch. ges. Physiol.*, 1925, **207**, 215; **209**, 611.

[2] Baumann, E., and Preusse, C., *Ber.*, 1895, **12**, 806.

[3] Baumann, E., and Schmitz, *Z. physiol. Chem.*, 1879, **20**, 586.

	Gm. Mercapturic Acid	% Yield
Baumann-Schmitz	2.764	15.3
Baumann-Preusse	2.508	14.0
Chloroform Extraction	3.372	18.5

The extraction method has not been perfected in detail, but may be described briefly. The urine is acidified with concentrated hydrochloric acid and let stand for 2 hours to liberate the mercapturic acid from the glycuronic acid complex. It is then extracted in a separatory funnel with 5 successive portions of chloroform, tracing the extraction by microscopic examination of a few drops of each extract. The combined extracts are evaporated, and the residue is taken up in very dilute ammonia, decolorized with carbon, and the filtrate acidified with hydrochloric acid. On standing overnight, an impure crystalline sediment is obtained. This is rubbed up with a few cc. of ether in a test tube, the ether is decanted from the crystals, and after another purification with dilute ammonia and carbon, almost colorless crystals are obtained. Modifications of this procedure are now being tested to eliminate certain undesirable features of the method.

Our results show that the Baumann-Schmitz method is unsuitable for application to alkaline rabbit urines. Also some doubt is thrown of the validity of interpreting any negative results with the above method as evidence that mercapturic acid is absent. Our findings on the synthesis of mercapturic acid in rabbits are significant in relation to a theory that the acid or basic character of the diet greatly modifies detoxication processes[1]. The oxidation of toluene and benzene and the conjugation of phenol, benzoic acid, and brombenzene have been cited in support of this theory. Braunstein and associates[4] showed that the oxidation of benzene and toluene, and the conjugation of phenol are independent of this dietary factor. Griffith[5] showed that the hippuric acid synthesis is not lessened on a basic diet as previously reported by Abderhalden[1]. Our findings seem to remove the last support of this theory, since the mercapturic acid synthesis is not inhibited by an alkaline diet.

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[4] Braunstein, Parschin and Chalisowa, *Biochem. Z.*, 1931, **235**, 311.

[5] Griffith, W. H., *J. Biol. Chem.*, 1925, **64**, 401.

6815

A Simplified Method for Determining Bile Salts in Bile.

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The metabolism of bile salts has received little attention due to the lack of a quantitative method which would give accurate results on small amounts of material. Five types of method have been tried: (1) using the Pettenkofer color reaction between cholic acid, furfural and concentrated sulfuric acid; (2) using separation of the bile acids and determination by weight; (3) stalagmometric methods depending on the property of bile salts to lower surface tension; (4) involving hydrolysis of the salts and subsequent determination of the amino nitrogen of the free taurine and glycocholic acid; (5) utilizing the fact that ferric salts of bile acids are insoluble.

Detailed criticisms of these methods by Hooper and Foster[1] and Schmidt and Dart[2] show that they are either too inaccurate or too laborious to be practical. One of the simplest recent methods is that of Szilard[3] who made use of a principle that has long been known, that bile salts are precipitated by ferric chloride,[4] and found that the iron in the precipitate is proportional to the bile salts present. In attempting to use this method and the modifications introduced later by Katayama,[5] it was found that the method was inaccurate and uncertain for small quantities of bile salts. Attempts to improve the technic have resulted in a new method which resembles that of Szilard only in its basic principle.

The new procedure is as follows: 5 cc. of bile are pipetted into a 100 cc. flask and diluted with 90 cc. of distilled water. Two cc. of 2 M (44%) calcium chloride, and 2 cc. of 4 M KOH are added and the whole is thoroughly mixed by shaking. Let stand for 10 minutes and then filter. The filtrate should be water clear and practically free from bilirubin, and if it is not, the bile is probably too concentrated and a greater dilution should have been made. Twenty-five cc. of this filtrate are pipetted into a 50 cc. beaker and 1 M HCl is added drop by drop until it is just acid to litmus. One cc. of 1.0% ferric chloride is then added and, on mixing, the bile salts are seen to precipitate in flocculent masses. When the amount of bile salt present is small, precipitation takes place slowly and one hour should be allowed. The contents of the beaker are then transferred to a 50 cc. Jena sintered glass filter funnel, grade 4, the surface of which is covered with a thin layer of asbestos fibers. The presence of the asbestos is important because without it the filtration process is too slow to be practicable. The filtration is done under moderate suction and the precipitate washed 4 times with 1 cc. portions of distilled water to remove the excess ferric chloride, and is then washed through into a 30 cc. test tube with 4 cc. portions of 95% ethyl alcohol. The alcoholic filtrate is mixed and sufficient additional alcohol is added to completely dissolve any precipitate which may have formed due to the presence of a small amount of water. One cc. of 2 M ammonium hydroxide is then added, and the tube is allowed to stand until the ferric hydroxide has completely precipitated, which usually requires from 5 to 15 minutes. The precipitate is then filtered out on the same filter, which has been previously washed with 1 M HCl and distilled water, and washed with 2 portions of distilled water of 1 cc. each and then washed through into a test tube with 1 cc. of 1 M HCl and two 1 cc. portions of 0.1 M HCl followed by 1 cc. of distilled water. The contents of the tube are then transferred to a 25 cc. volumetric flask, and the iron is determined after the method of Lyons,[6] which is as follows: 1 drop of thioglycolic acid is mixed with the flask contents and 2 M ammonium hydroxide is added drop by drop until a permanent red color develops, which is compared with a standard prepared in the same way using 0.2 mg. of iron. The standard iron solution is 0.703 gm. ferrous ammonium sulfate dissolved in a liter of 0.1 M HCl, so that 1 cc. contains 0.1 mg. of iron. The amount of iron in the sample is determined by comparing the 2 solutions in a colorimeter, and the amount of bile acid present in the original sample of bile is obtained by multiplying by the conversion factor and correcting for dilution, as indicated in the equation:

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[1] Foster, M. G., and Hooper, C. W., *J. Biol. Chem.*, 1919, **38**, 355.

[2] Schmidt, C. L. A., and Dart, A. E., *J. Biol. Chem.*, 1920, **45**, 415.

[3] Szilard, P., *Biochem. Z.*, 1926, **173**, 363.

[4] Hammarsten, O., Textbook of Physiological Chemistry, tr. by J. A. Mandel, New York, 5th ed., 1909, pp. 312-314.

[5] Katayama, I., *Arch. Int. Med.*, 1928, **42**, 916.

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[6] Lyons, E., *J. Am. Chem. Soc.*, 1927, **49**, 1916.

$$\frac{Rs}{Ru} \times S_f \times C \times \frac{Vt}{Va \times B} = \text{mg. bile acid in 1 cc. bile}$$

where Rs = reading of the standard
Ru = reading of the unknown
S_f = mg. iron in the standard
C = conversion factor (mg. bile acid per 0.1 mg. iron)
Vt = Volume of volumetric flask used
Va = Volume of aliquot of filtrate analyzed
B = Volume of bile taken

The amount of bile acid which corresponds to a given amount of iron can be calculated from the molecular weights of the acids, assuming that they combine with ferric iron stoichiometrically. In order to check this assumption, and at the same test the accuracy of the analytical method, samples of pure glycocholic acid and sodium taurocholate (generously supplied by Prof. C. L. A. Schmidt) were analyzed, with results shown in Table I. The findings justify the use of the conversion factor calculated from stoichiometric relations. The probable error of the method is about 1% when pure solutions of the salts in distilled water are analyzed.

TABLE I.

Material	Sample mg.	Iron Found mg.	Mg. Bile Acid per 0.1 mg. Iron	Calculated mg. Bile Acid per 0.1 mg. Fe
Glycocholic Acid (Pure)	5.0	0.198	2.52	2.51
" " "	3.6	0.145	2.48	2.51
Sodium Taurocholate (Pure)	6.3	0.206	3.06	2.98
" " "	8.6	0.275	3.12	2.98
" " (Practical)	9.4	0.226	3.28	2.98
" " "	7.8	0.235	3.32	2.98

In order to test the accuracy of the method when applied to bile, the recovery of added sodium taurocholate from dog bile was investigated with results indicated in Table II. These show an average recovery of added bile salt of 95%. This value has also been obtained in similar experiments with glycocholic acid.

TABLE II.

Bile cc.	Sodium Taurocholate mg. added	Iron Found mg.	Total Sodium Taurocholate mg.	Recovered Sodium Taurocholate mg.
1	0	0.412	12.3	—
1	0	0.396	11.8	—
1	10	0.725	21.6	9.6
1	10	0.715	21.3	9.3

The substances which ordinarily complicate bile salt determinations, such as cholesterol, amino acids, bile pigments, lecithin and lipoids, do not enter into consideration here since they do not form insoluble compounds with ferric chloride. Carbonates and phosphates which do form such compounds are removed by calcium chlorid and potassium hydroxide and the small amount which may remain is insoluble in alcohol and hence does not contribute to the error of the experiment. Precipitation of the pigments with calcium chlorid has been known for a long time but has not been used by other workers on this problem who instead employ charcoal in some form to adsorb the pigments. Kusui[7] has shown that such procedures also absorb an appreciable amount of the bile salts also.

Before this method can be used on bile from different animals, the relative proportions of the glycocholic and taurocholic must be known since the conversion factor of iron to bile acid depends on which acid is present. In the dog where only taurocholic acid is produced the problem is simple but in man where about equal amounts of both acids are found, an average value between the 2.9 of taurocholic acid and 2.5 of glycocholic acid, or 2.7, must be used.

The method was developed to study the bile acid excretion in patients with post-operative bile fistulas and particularly to study the effect of administering decholin (sodium dehydrocholate). It was found that decholin by mouth increased the amount of bile acids produced per hour in the fistula bile, reaching a high level in 3 to 5 hours after administration and then gradually falling off. The specific gravity and volume of the bile showed some increase.

Other samples of bile from various sources analyzed by this method show a great variation in concentration of bile acids. The highest concentration of bile salts was found in gall bladder bile from an ox which was 4.5%, and the least in fistula bile of a human which was from 0.1% to 0.4%.

This method can also be applied with no modification to the duodenal fluid obtained during a Lyon's drainage. Work is now in progress to adapt this method to the analysis of blood and urine.

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[7] Kusui, K., *J. Biochem.*, 1932, **15**, 399.

Illinois Section.

Billings Hospital, May 3, 1933.

6816

Oral Administration of Pneumococcus Antigen as a Therapeutic Agent in Experimentally Infected Rats.

H. E. MC DANIELS. (Introduced by Lloyd Arnold.)

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The object of the experiments reported here was to determine whether or not rats inoculated with lethal doses of pneumococci could be successfully immunized by means of oral vaccination begun after the introduction of the infecting dose. Various investigators[1] have demonstrated that young rats can be immunized against many lethal doses of virulent pneumococci by means of feeding certain pneumococcus antigens. Such experiments have all been along prophylactic lines; we have seen no reports of experiments testing the therapeutic value of pneumococcus antigens. Since it is possible to render young white rats resistant, by oral means, to comparatively large intraperitoneal or subcutaneous inocula of pneumococci, and since this resistance manifests itself within 24-48 hours, these animals were considered to be suitable for investigating the therapeutic value of oral immunization.

The method consisted of intraperitoneal injection of 100 lethal doses of Type I pneumococci into young white rats (60-100 gm.), followed by various antigenic preparations in single and also multiple doses. Certain rats were fed the antigen immediately after they received the injection of living organisms, and others were fed later during the course of their infection. Measured feedings were given by means of stomach tube and syringe. The antigenic preparations fed were tested for their efficiency on control animals;

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[1] Killian, H., *Z. f. Hyg. u. Inf.*, 1924, **102**, 279. Eguchi, C., *Ibid.*, 1925, **105**, 74. Kimura, R., *Ibid.*, 1927, **107**, 390. Ross, V., *J. Immunol.*, 1926, **12**, 219. Maëji, Y., *Acta Scholae med. univ. imp.*, Kioto, 1929, **12**, 295.

the minimum lethal dose was freshly determined and the lethality of the dilution actually used in the experiments was also checked on control animals. Rats dying during the course of these tests were all found to have pneumococci in the heart's blood.

As antigens in these tests, we used broth cultures which had undergone autolysis of 2 crops of pneumococci. This material was fed alone in some experiments; in others 2.0 cc. of egg-white[2] plus 3 cc. of autolysate was used. Bile was also used as an accessory substance with the autolysate in some of the experiments. This was sterile 5.0% ox bile, so that when 2 cc. was added to 3 cc. of autolysate, the resultant concentration of bile was 2.0%.

The results of single feedings are summarized in Table I.

TABLE I.

Rat	Live Pneumococci	5 cc. autolysate	Results	Remarks
1	100 M.L.D	Immediately	S	
2	” ”	”	D-2	
3	” ”	4 hr.	D-2	
4	” ”	4 ”	D-3	
5	” ”	8 ”	D-2	
6	” ”	8 ”	D-3	
7	” ”	12 ”	D-2	
8	” ”	12 ”	S	
9	” ”	24 ”	D-3	
10	” ”	24 ”	D-4	
11	” ”	32 ”	D-2	(Died before feeding)
12	” ”	32 ”	D-3	
13	” ”	not fed	D-3	(Control to indicate normal time of death)
14	” ”	” ”	D-3	
15	1000 M.L.D. given 48 hours after feeding		S	(Control of efficiency of autolysate)
16	100 M.L.D. given 48 hours after feeding		S	

S = survived. D-2 = died second day.

A similar set-up, using 32 rats, was tested with a single dose of the egg-white and bile reinforced antigens described above. The control animals, tested 48 hours after feeding each antigen mixture, where found to be resistant to 10 times the dose used in the test animals. All of the test animals died from the second to the fourth day.

Ten rats, inoculated with 100 M.L.D. and fed 5.0 cc. of autolysate every 2 hours (5 doses over a period of 10 hours), beginning immediately after the inoculation, all died within 2-4 days.

From these experiments it seems that although the course of experimental pneumococcus peritonitis in rats is of 2-4 days' duration, a time which is adequate for the development of orally stimulated immunity in control rats, the infection proceeds to a fatal termination in spite of the immediate institution of this treatment. The survival of 2 rats out of the 60 animals used in these experiments is to be attributed to individual differences in the rats; this factor of variation ordinarily appears in such experiments to an even greater extent than in this case. The immune response in already infected rats apparently is inhibited so that oral immunization is ineffective as a therapeutic agent under these conditions in these animals.

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[2] McDaniels, H. E., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 587.

6817

Cerebral Action Potentials.*

R. W. GERARD, W. H. MARSHALL AND L. J. SAUL.
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The following observations, some confirmatory, based on experiments on 8 cats and 2 monkeys, were made during an exploration of the cerebral cortex and underlying structures for spontaneous and evoked activity. Anatomical relations given are only approximate; the work is being continued with the aid of a Horsley-Clark stereotaxic instrument. Action potentials picked up by an Adrian-Bronk electrode, amplified, and fed into a loud speaker and high-voltage cathode ray oscillograph, were the index of activity. Numerous controls have convinced us that extraneous pick-up did not confuse true action potentials in the phenomena described. For example, during penetrating movement of the needle of less than 1 mm. there appeared successively: nothing, strong auditory responses, nothing, strong optic responses, nothing. Clearly, responses may be highly localized, and a non-specific spread is excluded.

Auditory responses were obtained from the temporal cortical surface, projection tracts, auditory thalamus, and lower structures in the monkey, from all but cortex in the cat. With the electrodes on a large tract, responses were obtained to stimuli near the human threshold. Different sounds (*e. g.,* watch tick and voice) and pitches are recorded most strongly at different needle positions, indicating a spatial separation of impulses set up by different pitches. Similarly, a fairly sharp di-, tri- or polyphasic wave appears on the

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* Aided by a grant from the National Research Council to R. W. G.

oscillograph for each watch tick. The form differs from R. to L. ear for one needle position, and for one ear at different needle positions.

Considerable discharge was usually present in the pathways even without deliberate sound stimulation. In the monkey, a stimulus at 660 d.v. gave a standing wave of the same frequency on leading from auditory tracts, a completely asynchronous discharge, stronger at the start, from the cortical surface. In the cat and monkey, melodies could usually be recognized on leading from auditory tracts, although words were garbled.

Optic responses were obtained from the cortical surface, radiations, optic thalamus and tracts in the monkey and cat. (One experiment from the frog's optic lobe.) Responses to slight finger movements several inches from the eyes were obtained in diffuse daylight, sensitivity being greatest in dark-adapted eyes. Tracts gave maximum responses usually at on and off, though sometimes asynchronous or even partially synchronized discharges continued undiminished during the entire light period. A marked after-discharge was often present, sometimes as long as 5 seconds. Cortical responses were entirely asynchronous.

In the cat, a very large cortical area yielded responses, best in the occipital pole, falling off slowly anteriorly, more rapidly laterally, but detectable well into the frontal region. Localization appeared sharper in the monkey, both as to total optic area and definition of the margin. The response diminished, often to zero, as the electrode penetrated the cortex, then increased to a maximum intensity at 3-4 mm. depth in the cat, 7 mm. in the monkey. Responses from these underlying tracts were distinctly sharper (more synchronous) than from the superjacent cortex. Bilateral responses were the rule. In several instances optic and auditory responses were obtained from the same electrode position, and in such cases the response to a regular auditory stimulus seemed to be increased when a light was flashed into the eyes.

Somaesthetic responses were obtained from the post-central gyrus of the monkey and showed the anticipated localization. Thus, gentle stroking of the hair of each limb in turn gave a response in the medial portion of the gyrus only from the crossed leg, in the lateral portion only from the crossed arm. Presumable proprioceptive stimulation by gently flexing the toes gave responses in the foot region of the pre-central gyrus. A more intense continued activity was obtained from the post-central than the pre-central gyrus.

"Spontaneous" activity was regularly obtained in the absence of

deliberate stimulation, varying from a continuous background through waves to sharp peaks. Though some of these potentials were surely evoked by unidentified afferent impulses, others probably represent (spontaneously) changing cell potentials. They were often well localized, intense, relatively slow in frequency and duration of each wave, and were reversibly and fairly continuously depressed by ether. Evoked activities, such as optic and auditory, showed little change as ether was pushed, until they sharply disappeared; and returned in the same way. The slow potential waves, on the contrary, failed and returned gradually, changing both amplitude and frequency, and light narcosis led to augmentation rather than depression. In several cases the "spontaneous" activity suddenly increased (needle not moved), to be followed in a few seconds by general struggling. Pure CO_2 inhalation reversibly depressed activity to zero, after an early increase.

Rhythmic discharges were obtained, mainly from thalamic and hypo-thalamic regions, but also (and especially in the monkey) from frontal and occipital lobes. (Large semirhythmic waves were obtained from the cerebellum.) These varied from slow waves, almost of sine shape with "overtones", at 40-80 a second and standing on the oscillograph, to sharp, short, diphasic spikes, varying from 10 to 100 per second and of regular frequency. Intense high frequency (1000 or more) semi musical "howls" were given by basal regions. Many of these rhythmic discharges waxed and waned in intensity over intervals of a few seconds to 3 minutes, probably as individual units became more or less synchronized. In one case, light in the contralateral eye inhibited a strong, continuous discharge. Discharges were obtained from the hypo-thalamic region synchronous with the heart beat and with respiration. Traction on the liver produced long lasting activity in the same vicinity. Though it is impossible to describe these various rhythmic discharges, many possess a distinct individuality and can be recognized easily over the loud speaker, distinguished sharply from one another, and identified from animal to animal.

It is interesting that animals under avertin anesthesia, presumably entirely unconscious and yielding no reflex response to painful stimulation of the legs, no knee jerk and but a slight pupillary reflex to light, should still give marked electrical responses to these same stimuli from the cortex itself. The question of the relation of neural activity to conscious states is thus brought into focus.

6818

Effect of Growth-Promoting Extracts of Bovine Anterior Hypophysis on Hypophyses of Castrated Albino Rats.*

A. M. TARGOW. (Introduced by F. C. Koch.)

From the Department of Physiological Chemistry and Pharmacology, University of Chicago.

In view of the reports of Smith[1] and of Evans and Simpson[2] on the prevention of the peripheral action of the gonad-stimulating complex of the anterior pituitary by the simultaneous administration of anterior pituitary growth-promoting extracts, it was thought that the latter might have some influence on the increase in gonad-stimulating potency of the anterior pituitary which has been shown to follow castration.[3]

A growth-promoting extract was prepared from bovine anterior lobe tissue according to the method of Bugbee, Simond, and Grimes.[4] The extract contained also a slight amount of gonad-stimulating substance since a single intravenous injection of 7 cc. produced ovulation in the rabbit, and 0.5 cc. daily for 6 days caused a slight increase in weight of the ovaries and uteri of immature female rats.

The work of Evans and Simpson was confirmed on a group of 12 immature female rats, 4 litters of 3 littermate sisters each. Two females from each litter received 3 glands apiece from littermate male donors daily for 4 days; and to one of these females was administered simultaneously 0.5 cc. of the growth-promoting extract twice daily, subcutaneously, for the 4 days. The females were killed 48 hours after the last implantation, the ovaries and uteri were removed and weighed. The largest ovaries and uteri were found in the group receiving the implantations alone; in the group receiving the implantations plus the extract, the organs were definitely smaller than in the preceding group, indicating a nullification of the action of the implants; yet the organs were definitely larger than in the case of the untreated control group, hence the nullification of the

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* This work was supported in part by Biological Grant No. 12 of the Rockefeller Foundation.

[1] Smith, P. E., *J. Am. Med. Assn.*, 1927, **88**, 158.

[2] Evans, H. M., and Simpson, M. E., *J. Am. Med. Assn.*, 1928, **91**, 1337.

[3] Engle, E. T., *Am. J. Physiol.*, 1929, **88**, 101; Evans, H. M., and Simpson, M. E., *Am. J. Physiol.*, 1929, **80**, 371.

[4] Bugbee, E. P., Simond, A. E., and Grimes, H. M., *Endocrinology*, 1931, **15**, 41.

action of the implants was not complete, as reported by Evans and Simpson. This difference can, of course, be explained on the basis of relative dosage.

The extract having thus been shown to be essentially similar to the one employed by Smith and by Evans and Simpson, the following experiment was set up: A total of 120 male albino rats was accumulated, of 30 groups, 4 littermates to a group. Each group of littermates was weaned on the 21st day of life and castrated on the same day. Injections were started the same day, or the day following, and were given subcutaneously, 0.5 cc. twice daily. One animal in each group received the growth-promoting extract; the second animal received the inactive beef-muscle extract; the other 2 animals remained as untreated controls. The injections were continued for about 40 days, or until the animals were approximately 60 days old. At this time, 3 animals from each group were killed. These consisted of the one receiving the growth-promoting extract; the one receiving the beef-muscle extract; and that one of the untreated controls which weighed the most; this latter control thereby served as an age control. The smallest untreated control was allowed to grow until it had reached approximately the same gross weight as its littermate receiving the growth-promoting extract, at which time it was also killed; this control thereby served as a weight control.

When the hypophyses were removed, it was found that both on an absolute and relative weight basis, both the anterior and posterior lobes of the glands from the animals receiving the growth-promoting extract were smaller than in the case of the glands from the 3 control groups; whereas the weights of the glands in the control groups did not differ significantly from one another. Nevertheless, on implantation into immature females, the anterior lobes from the "growth" treated group showed no significant lessening of potency to stimulate the genital system as indicated by ovarian and uterine weights. The significance of the data was arrived at by statistical treatment by the method of paired comparisons.[5]

It is concluded that growth-promoting extracts of the nature described above, when injected for approximately 6 weeks following castration do not prevent the increase of gonad-stimulating potency of the hypophysis, as revealed by the implantation technique, yet do cause a marked diminution in weight of both the anterior and posterior lobes.

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[5] Fisher, R. A., Statistical Methods for Research Workers, 3rd ed., 1930, 104.

6819

Results of Inoculation of Poliomyelitis Virus into Mice.

W. J. NUNGESTER.

From the Department of Bacteriology, Northwestern University Medical School.

It has been shown[1] that organisms suspended in commercial gastric mucin and injected intraperitoneally into mice have greater effect on the host than an inoculum of such organisms suspended in saline. With this in mind it was decided to try to produce an infection with the virus of poliomyelitis in mice with the aid of mucin.

The virus containing glycerinated monkey cord, kindly furnished by Dr. Paul Hudson, was ground with sand and diluted so that the inoculum contained about 1% of the ground cord. Mucin* was sterilized by boiling for 3-4 hours in alcohol, decanting the alcohol, drying the mucin *in vacuo* at 50° C. and finally making a 5% emulsion of it in saline.

Mice were inoculated with various lots of virus containing cord suspended in saline or mucin. Control mice received an inoculum of mucin alone or in some instances ground glycerinated normal dog cord suspended in mucin. One cc. was inoculated in each case.

Seventy-six mice have been inoculated intraperitoneally with various batches of virus suspended in mucin emulsion. Of these 13 developed a muscular weakness which tended to affect particularly the hind legs and tail. Two mice developed a flaccid paralysis, one of the left side and the other of the right hind leg. Deaths have occurred in 22 mice in from 1 to 41 days (average 9.4 days) following inoculation. In 8 of these animals the lungs, which partially collapsed on opening the chest, were red to dark red. Heart blood of these mice inoculated to blood agar gave no evidence of infection.

Thirty-nine mice were inoculated with virus suspended in saline. Weakness was noted in 4 of these in from 2 to 9 days. Death occurred in 6 mice in from 1 to 16 days (average 5.8 days). Flaccid paralysis of the extensor muscles of the right hind leg was noted in one. This instance of paralysis as well as of the cases of paresis that developed and 5 of the 6 deaths in the saline group occurred with a lot of virus material which appeared to be particularly virulent for mice. This material killed 3 out of 3 mice inoculated with it and mucin in 1 day, and 3 out of 3 mice in the saline group in 1, 2, and 3 days respectively. Also 2 out of 4 mice given 1:100 of the usual inoculum in saline died in 9 days. Blood cultures of the heart blood of the saline animals failed to reveal an infecting organism. A spreading type of growth covered the surface of plates receiving the inoculum from the mucin mice in this test, which was interpreted as a post mortem invasion or contamination.

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[1] Nungester, W. J., Wolf, A. A., Jourdonais, L. F., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 120.

* Dr. S. Fogelson suggested the use of and supplied washed gastric mucin which is now used in place of the commercial gastric mucin previously employed.

Mucin alone or mucin and emulsified glycerinated normal dog cord has been inoculated into 16 mice, one of which died in 1 day. No paresis or paralysis developed in the other animals.

Seven attempts to establish serial infections in mice have been made. Three of these efforts resulted essentially in failure. However from the cords of mice dying in the 4th generation of series I, suggestive histological evidence for an inflammation of the spinal cord has been uncovered by Dr. Weil in a study of material from this work. A filtrate of the ground organs of these mice when injected intraperitoneally with mucin into 2 mice produced weakness in 6 days and death in 18 days in one mouse and weakness at 17 days but no death in the other.

Conclusion. Although the work presented does not prove conclusively that mice can be infected with poliomyelitis virus, the evidence, when considered together with the histological study of the material by Dr. Weil, appears to warrant further consideration of the mouse as an experimental animal for the study of this disease.

6820

A New Type of Centrifuge Tube for Preparation of Blood Serum for Accurate pH Work.

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From the Department of Physiological Chemistry, University of Chicago.

The conventional methods for the preparation of blood serum for pH determination involve the following steps:[1] the blood is drawn into centrifuge tubes under oil; the oil is displaced with wax or with a rubber stopper; after centrifuging, the wax or rubber stopper is again replaced with oil and the serum lying between the oil and the cells is withdrawn with a pipette which also contains some oil. Aside from being time-consuming this procedure has a primary difficulty in that the contact of the serum with the oil brings about changes in the CO_2 tension and thus in the pH of the serum. This difficulty has been overcome in part by using oil having the same CO_2 tension as the blood. Often, however, the CO_2 tension of the blood is not known beforehand and this remedy is at best time-consuming and approximate.

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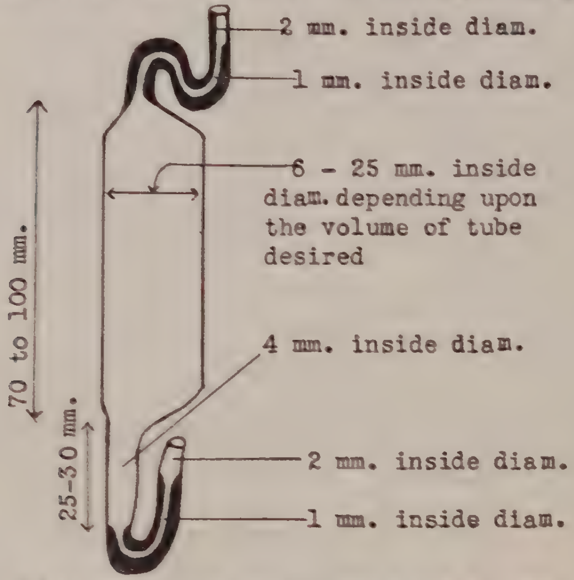
[1] Austin, Cullen, Hastings, McLean, Peters, and Van Slyke, *J. Biol. Chem.*, 1922, **54**, 121.

A centrifuge tube is here described which enables one to prepare serum from blood without contact with anything except glass and mercury. The accompanying sketch shows a tube with an S shaped capillary at the top and a U shaped capillary at the bottom. For filling the centrifuge tube with blood a T-tube with stopcock below the side-arm is attached to the upper capillary and the hypodermic needle is attached to the upper end of the T-tube, both through rubber connections. A rubber tube leading to a mercury leveling bulb is attached to the lower capillary through which the centrifuge tube is filled with mercury. Care must be taken to sweep all bubbles of air out of the S capillary. The mercury level is put a few millimeters above the side-arm of the T-tube and the stopcock below the side-arm is closed. The needle is now inserted into the blood vessel and about one cc. of blood is caused to flow through the side-arm of the T-tube above the stopcock, sweeping any air out ahead of it. The side-arm of the T-tube is then closed and with the mercury leveling bulb in a lower position, the stopcock is opened



and the blood thus caused to flow into the centrifuge tube. The rate of flow of the blood may be controlled by the height of the mercury leveling bulb and also by means of the stopcock.

Blood is drawn in until the mercury blood meniscus is in the lower part of the 4 mm. tube, that is, about 2 cm. above the bottom of the tube. Mercury is then run in through another side-arm in the T-tube at the top until the S capillary is filled with mercury. Any excess mercury admitted here escapes below without displacing any significant amount of blood. The stopcock is then closed, the leveling bulb tubing removed below, and then the rubber tubing removed above. The tubing above must not be removed before the leveling bulb below is removed or the pressure or suction of the mercury in the leveling bulb will remove blood from the tube.

The tube is in remarkably stable gravitational equilibrium, although it has only mercury seals above and below. The mercury in the open capillary arm of the U bend is about 1 cm. higher than the mercury in the other 4 mm. arm and this effectively balances the weight of the 10 cm. column of blood. The tubes may be centrifuged at high speed and the serum separates rapidly and effectively.

For transferring the serum, the centrifuge tube is clamped to a stand and a suitable delivery tube is attached above. A mercury leveling bulb with stopcock or clamp in the rubber tubing is attached to the U tube below and the serum is expelled above by mercury from below. By controlling the rate of inflow of the mercury by means of the stopcock in the leveling bulb line, the serum may be expelled very slowly and thus any stirring up of the cells is entirely avoided and a more quantitative transfer of serum can be made than is possible in any other kind of tube. Although the blood clots, the 4 mm. inside diameter is wide enough to allow the mercury to flow in freely while the serum is transferred.

The washing of these tubes is sometimes difficult since the clot cannot be removed directly through the capillary openings. If a vigorous stream of hot water is sucked through the tube within an hour after centrifuging, the clot frequently disintegrates and can be removed at once. When the clot remains we have found it best to fill the tube with normal NaOH and to set it in a warm place for several hours, later washing with a stream of hot water. Two such washings with NaOH have never failed to remove the clot.

These tubes have been used in sizes of 3 cc., 15 cc., and 40 cc. With the 15 and 40 cc. sizes the results on the pH's of triplicates drawn simultaneously agree to within 0.005 pH.

6821

Carbohydrate Utilization by Bacteria Without Evident Acid Production.

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From the Department of Bacteriology, Northwestern University Medical School.

There has been developing recently an increasing realization that bacteria may utilize carbohydrates without an increase in hydrogen-ion concentration. We have undertaken to determine the extent of this type of carbohydrate utilization by examining a number of microorganisms representative of a wide range of bacterial groups, testing each organism in those sugars in which it showed no acid according to the usual indicator method. A series of sugars was added to (1) sugar free beef infusion broth and (2) 2% peptone 0.5% sodium chloride solution as basic mediums, to make 0.4% solutions of the monosaccharides and 0.8% of the disaccharides. The organisms were grown in these sugar mediums at 37° C. for 2-3 weeks (lactose for 3 months, also) and then the mediums were analyzed quantitatively for sugar by a copper reduction method.[1] In most cases, when the use of sugar was apparent only by quantitative analysis, the experiment was repeated and the hydrogen ion concentration determined daily. In doubtful instances, the experiment was repeated in these mediums buffered with phosphates.

Table I shows the sugars employed and those organisms with which the utilization of sugar was always accompanied by evident acid production. Table II shows those organisms which utilized one or more sugars according to quantitative analysis while concurrently the medium increased in alkalinity. In such instances, subcultures to the appropriate carbohydrate indicator agar plates failed to exhibit acid production, except regularly with *Sarcina lutea* in maltose, and irregularly with *B. pyocyaneus* in xylose. There were no manifest evidences of colonial dissociation. The sucrose, mannitol, indol, negative *B. dysenteriae* Shiga cultures were the only ones to show a decrease in pH (a rather sudden drop to about pH 6.0 after remaining at about 6.8 for 10-15 days). The entire typhoid-paratyphoid group have given irregular evidences of slight lactose utilization, but nothing conclusive, even after 3 months' growth.

The tables indicate that for most organisms, utilization of carbo-

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[1] Stiles, H. R., Peterson, W. H., and Fred, E. B., *J. Bact.*, 1926, **12**, 427.

TABLE I.

Organism	Strains	Dextrose	Mannose	Fructose	Galactose	Xylose	Arabinose	Lactose	Maltose
Staphylococcus	5	+	+	+	+	—	—	+	+
Staph. citreus	1	—	—	—	—	—	—	—	—
Streptococcus	1	+	+	+	+	+d	—	+	+
M. tetragenus	1	+	+	+	+	—	—	+	+
M. catarrhalis	1	—	—	—	—	—	—	—	—
B. alcaligenes	1	—	—	—	—	—	—	—	—
B. dysenteriae Flexner	2	+	+	+	+	—	+	—	+
B. typhosus	4	+	+	+	+	+	—	—	+
B. paratyphosus alpha	4	+	+	+	+	—	+	—	+
B. paratyphosus beta	2	+	+	+	+	+	+	—	+
B. cholerae suis	1	+	+	+	+	+	+	—	+
B. enteritidis	1	+	+	+	+	+	+	—	+
B. icteroides	1	+	+	+	+	+	+	—	+
B. morgani	1	+	+	+	+	—	—	—	—
B. pullorum	1	+	+	+	+	+	+	—	—*
B. avisepticus	1	+	+	+	+	+	+	—	+
B. diphtheriae	2	+	+	+	+	—	—	—	+
B. diphtheriae (Park 8)	1	—	—	—	—	—	—	—	—
B. hofmanni	1	—	—	—	—	—	—	—	—
B. anthracis	3	+	I	+	—	—	—	—	+

+ = clearly evident acid; d = delayed utilization; — = no sugar utilization according to quantitative analysis or indicator; * = irregular; I = inconclusive at present.

TABLE II.

Organism	Strains	Dextrose	Mannose	Fructose	Galactose	Xylose	Arabinose	Lactose	Maltose
Sarcina lutea	1	A_p†	C_3	I	—	I	—	I_3	A_p*
B. pyocyaneus	2	C_p	C†	C_p	C†	C_3*	C	I_3	A_p†
B. leprae	2	B_p	B_p	B_p	I	C_p	A_p	$—_3$	—
B. smegmatis	1	B_p	C_p	C_p	C_p	C_p	C_p	$—_3$	—
B. phlei	2	+	+	+	+	C_p	C_p	+	+
B. mesentericus	1	+	C_p	+	B_p	B_p	C_p	B	—
B. subtilis (Cohn)	1	+	+	+	$+_d$	C_p	+	C	+
B. proteus	7	+	—	+	+	+	$—_3$	A	+
B. dysenteriae Shiga	2	+	+	+	+	—	—	C_3*	+

+ = clearly evident acid accompanying utilization.
A = 10-20% sugar utilized; B = 20-50%; C = 50-100%.
p = daily pH showed gradual rise; d = delayed utilization.
3 = 3 months culture (2 weeks inconclusive).
I = inconclusive; * = see text; † = inconstant.
— = no evidence of utilization by quantitative analysis or by indicator.

hydrate is accompanied by clearly evident acid formation, but that (as others have pointed out) certainty as to utilization of carbohydrate can not be attained by methods relying alone upon such formation. This work is being continued.

6822

Skin Tests for Sensitivity to Virus of Poliomyelitis.

PAUL H. HARMON, JAMES A. HARRISON AND GRAHAM KERNWEIN.
(Introduced by Lester R. Dragstedt.)

From the Department of Surgery, Division of Orthopedics and the Milbank International Fund for the Study of Infantile Paralysis, Department of Hygiene and Bacteriology, University of Chicago.

Early investigators[1, 2] denied that the convalescent state in monkeys recovering from poliomyelitis was accompanied by cutaneous hypersensitiveness. Aycock[3] has stated that he has been unable to detect skin hypersensitiveness to monkey passage virus in human convalescents. Both Aycock and Kagan[4] and Stewart and Rhoads[5] while actively immunizing monkeys by the intracutaneous method, failed to note cutaneous allergic reactions. Recently, Jungeblut[6] has found that while cutaneous reactions of hypersensitiveness are lacking in convalescent monkeys, there is a high degree of generalized hypersensitiveness in these animals as judged by the occurrence of an immediate thermic response to the introduction of virus either intracerebrally or subcutaneously. Such a response was lacking in animals that had been uninfected. According to this author[7] the reverse situation obtains in man, as he observed a definite specific cutaneous reaction to an emulsion of virus-bearing monkey spinal cord in each of 27 human individuals with residual paralysis due to poliomyelitis. Sabin[8] has failed to find evidence of an allergic skin reaction in normal adults or in human convalescents either recent or of long stand-

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[1] Römer, P. H., ''Die Epidemische Kinderlähmung'', Berlin, Springer, 1911.

[2] Leiner, C., and von Wiesner, R., *Wien. klin. Wchnschr.*, 1909, **46**, 2331.

[3] Aycock, W. L., personal communication.

[4] Aycock, W. L., and Kagan, J. R., *J. Immunol.*, 1927, **14**, 85.

[5] Stewart, F. W., and Rhoads, C. P., *J. Exp. Med.*, 1929, **49**, 959.

[6] Jungeblut, C. W., *J. Exp. Med.*, 1931, **53**, 159.

[7] Jungeblut, C. W., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1072.

[8] Sabin, A. B., quoted by Harrington, H., in ''Poliomyelitis'', Baltimore, The Williams and Wilkins Company, 1932, 126.

ing, when tested with fresh heat inactivated preparations of monkey virus, human virus and normal monkey cord.

Since one of us (J. A. H.) had prepared a concentrated and purified virus from which a large share of proteins, phospholipins and lecithins had been eliminated, we decided to test the cutaneous reactions of poliomyelitis convalescents with this product. Using a similarly prepared concentrate of normal monkey spinal cord as a control, we have carried out intradermal injections upon 17 children having residual paralysis from poliomyelitis. These children varied from 2 to 19 years of age. The acute attack of poliomyelitis had been passed from 5 months in 2 instances to 14 years in one instance, the majority being fewer years remote from the acute stage of the disease. Tests were also done upon the same group of convalescents with crude 5% monkey virus and with a 5% suspension of normal monkey spinal cord. All preparations were heated upon 2 occasions to 55°C. for 30 minutes, a temperature that had been demonstrated to be well beyond the thermal death time for poliomyelitis virus.[9] Another group of 23 non-poliomyelitic children convalescent from miscellaneous orthopedic complaints received intracutaneous inoculations of all 4 preparations.

That none of these preparations contained viable virus was demonstrated by intracerebral injection of 1.0 cc. of both the concentrated and unconcentrated virus into unused *Macacus rhesus* monkeys by the intracerebral method. No paralysis was observed in either animal during one month subsequent to inoculation. All preparations were shown to be sterile by the usual bacteriological methods.

Very few cutaneous reactions were observed with crude poliomyelitis virus preparations and when present there was always a cutaneous reaction to the corresponding control injection of normal monkey spinal cord emulsion. One-third of both groups of children demonstrated a definite cutaneous erythema reaching its height 24 hours after injection when the concentrated preparations were used. Again there were no differences observed between the virus cord concentrate and the similar preparation of normal monkey spinal cord. No immediate cutaneous reactions were observed.

Eight children convalescent from poliomyelitis were also tested in groups of 2 to each of the 4 preparations given subcutaneously. Following inoculation of 0.5 cc. by this method the rectal temperature of these 8 children was followed by 2 hourly periods for 24 hours. No deviations from a normal temperature were observed in any instance and no induration developed about the site of inoculation.

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[9] Shaughnessy, H. J., Harmon, P. H., and Gordon, F. B., *J. Prev. Med.*, 1930, **4**, 149.

6823

On the Ionization of Calcium Citrate.

FRANKLIN C. MCLEAN, A. BAIRD HASTINGS, LILLIAN EICHELBERGER AND JAMES LOWELL HALL.

From the Physiological Laboratory and the Lasker Foundation for Medical Research, the University of Chicago.

The fact that the addition of sodium citrate to calcium containing solutions reduces the concentration of ionic calcium is well known. The nature of the combination between calcium and citrate has not been definitely established. It has been sometimes thought that a neutral compound $Ca_3(citrate)_2$, exists, but this is not consistent with the evidence that the combination between calcium and citrate is negatively charged.[1]

For this reason the frog heart preparation which had been shown to be sensitive to calcium ion changes under controlled conditions was used to assay the calcium ion concentration in solutions of varying total calcium and citrate content.

The results of such experiments are shown in the accompanying figure. The experimental points show the concentrations of calcium and citrate present in solutions which are iso-active with corresponding solutions containing no citrate, but containing a certain concentration of calcium ions. Each series of symbols represents physiologically isoactive points. The relationship for a given concentration of calcium ions appears to be linear for the limits within which this technique is accurate, *i. e.*, calcium ion concentrations from 0.3 to 1.2 millimols per liter. This has been found to be true when the total calcium concentration is as high as 30 millimols per liter.

These results are consistent with the interpretation that (1) calcium combined with citrate does not affect the amplitude of contraction of the frog heart and (2) that one mol of calcium combines with one mol of citrate to form a calcium citrate compound

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[1] Greenberg, D. M., and Greenberg, L. D., *J. Biol. Chem.*, 1932, **99**, 1.

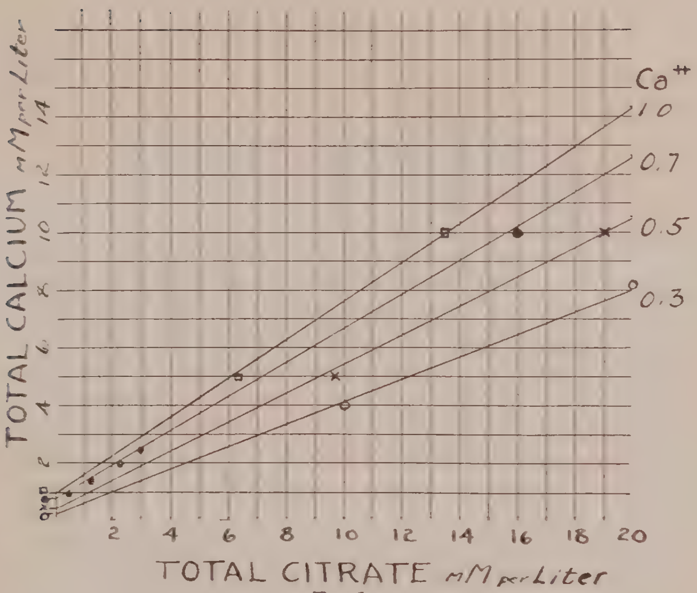

FIG. 1.

carrying one negative charge. The straight lines in the figure fit the equation,

$$\frac{[\mathrm{Ca}^{++}] \times [\mathrm{citrate}^{\equiv}]}{[\mathrm{Ca\ citrate}^{-}]} = 10^{-3.31}.$$

The same problem has also been studied chemically by equilibrating salt solutions containing citrate with calcium carbonate and calculating the calcium ions from the solubility product constant of the solid phase. These experiments led to the value $10^{-3.37}$ for the ionization constant of the calcium citrate when equilibrium was approached from the condition of undersaturation.

6824

Studies in Local Immunization of the Lungs of Rabbits with Pneumococcus Type 1.

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From the Department of Pathology, University of Chicago.

Accumulating evidence indicates that an enhanced resistance to bacterial infection may be produced locally as well as generally. To gain further information as to the mechanism of this local immunity we have immunized rabbits intrapulmonically, subcutaneously and intravenously with a pneumococcal vaccine (Type I) and at a later period have tested the resistance of these animals to living pneumococci introduced intratracheally. We have also attempted to determine the fate of the pneumococci, both as to their retention within the lungs and their rates of appearance and disappearance in the blood stream.

Adult male rabbits weighing from approximately 3000 to 4000 gm. were immunized daily for from 4 to 6 days by injections of one cc. of a formolized vaccine of pneumococci. The vaccine was prepared by growing the pneumococci in Kolle flasks and adding 0.2 cc. of formalin to each 100 cc. of the bacterial growth suspended in 0.85% solution of sodium chloride. The intratracheally treated animals were immunized by means of a curved metal tube introduced into the trachea through the mouth; the others were immunized by subcutaneous and intravenous injections of similar amounts of the vaccine. The turbidity of the vaccine was between that in tubes 1 and 2 of the McFarland nephelometer. From 5 to 15 days after the final treatment the animals were infected in groups of 4, consisting of one normal rabbit and an animal immunized by each of the 3 ways mentioned. The 24-hour growth from a blood agar slant of a living pneumococcus culture, Type 1, was suspended in 8 cc. of sterile broth, and 2 cc. was then introduced intratracheally into each of the 4 animals through the metal tube inserted into the trachea. Blood was obtained by cardiac puncture at 5, 10, 15, 30, 45, and 60 minute intervals after infection and cultures made by plating duplicate amounts of one cc. with veal infusion agar, pH 7.6. The presence and numbers of bacterial colonies per cc. of blood indicated the relative degrees of retention of the pneumococci within the lungs and also the rates of appearance and disappearance of the microorganisms in the blood stream. The fatality rates for each group were recorded at the end of one week.

Fifty-one animals were infected intratracheally with the living pneumococci, 17 normal rabbits, 17 immunized intratracheally, 9 subcutaneously and 8 intravenously. Many bacterial colonies appeared in the blood cultures of the normal rabbits as well as in those immunized subcutaneously and intravenously within from 5 to 15 minutes after infection, whereas in most instances, during the entire 60-minute period, none or only a few appeared in the blood cultures of the animals immunized intratracheally. The bacteria disappeared rapidly from the blood stream in the intravenous and subcutaneously immunized animals and much more slowly in the normal rabbits. All of the normal rabbits died within from 12 to 24 hours and at autopsy the lungs were hyperemic and edematous. Three of the subcutaneously immunized and one of the intravenously immunized animals died within 24 hours and their lungs at autopsy resembled those of the normal animals. Only one of the intratracheally immunized rabbits died, and this animal survived for 4 days. The lungs at autopsy were definitely consolidated with their surfaces roughened by fibrin. No appreciable amounts of blood were found in the pericardial cavities of any of the animals. All of the other rabbits survived.

In addition to these experiments, others were performed to show the fatality rates alone. The rabbits were immunized intratracheally and subcutaneously as described above, and their resistance to living pneumococci introduced was compared with normal animals. Seventy animals in all were tested, of which 26 were normal, 26 intratracheally immunized, and 18 subcutaneously immunized. All of the normal rabbits, and 3 of those subcutaneously immunized died, whereas only 2 of the intratracheally immunized animals died.

6825

Control of the Bill Color of the Male English Sparrow by Injection of Male Hormone.*

WARREN N. KECK. (Introduced by Emil Witschi.)

From the Laboratory of the Department of Zoology, University of Iowa.

The writer recently reported[1] the effect of castration upon the bill color of the male English Sparrow. In brief, the effect is a gradual disappearance of the pigment until the characteristic ivory-colored bill of the capon is established by the end of the sixth week.

A set of experiments was planned to test the possible restoration of the black color by injection of male hormone into sparrow capons. The male hormone was extracted with chloroform from the urine of men of approximately 20 years average age. The preparation was standardized in rat units using the seminal vesicle test as the criterion of standardization. Fourteen capons that had been castrated for 6 months were divided into 4 groups. Two individuals served as controls and the remaining 12 were divided into 3 groups of 4 each.

The individuals of group I received 3/4 of a rat unit daily for 25 days; those of Group II were given 1/6 of a rat unit daily for the same period; individuals of Group III each received 1/15 of a rat unit daily for 25 days. A noticeable change in bill color was evident after 8 days of injection. At the end of the experimental period the bills of the individuals receiving the high dosage were black like that of normal males during the breeding season. The capons receiving the low dosage had the anterior half of the bill blackened, but the basal portion remained light. Group II showed an intermediate condition. While the entire bill was dark in color, the black was not as intense as in Group I. The deposition of pigment always begins at the apex of the bill and proceeds in a posterior direction, thus indicating a gradient of threshold values, falling off from the base toward the tip of the bill.

The dosages of hormone given were only fractions of that necessary to produce an effect upon the seminal vesicles of the rat. Furthermore, a definite response can be obtained within 10 days which equals the time necessary to standardize in rat units.[2] While the comb-growth response of capons to male hormone requires less time, many factors are involved such as light and accurate measurement that render its routine application rather difficult. It is also easier and much less expensive to maintain a sparrow colony in the average laboratory.

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* Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council; grant administered by Dr. Emil Witschi.

[1] Keck, W. N., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 158.

[2] Hansen, I. B., *Endocrinol.*, 1933, **17**, 163.

The results of this study, therefore, suggest the use of the bill color of the sparrow capon for the standardization of extracts of male hormone. More extensive experiments are planned to test its practicability.

6826

A Functional Study of the Nodose Ganglion of the Vagus with Degeneration Methods.*

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In 11 adult cats, the vagus (5 on the left, 6 on the right) was cut at its exit from the jugular foramen above the nodose ganglion. In 5 experiments, the superior cervical sympathetic ganglion was also removed. Approximately 15 days' degeneration was allowed. The animals were anesthetized with urethane or ether, the carotid (in some cases the femoral) artery was cannulated and prepared for blood pressure tracings. In some experiments, movements of the thorax were also recorded as an index for the rate of respiration. The nerves were stimulated with rapid faradic stimuli delivered through platinum electrodes which were connected to a Harvard inductorium (approximately 2 amperes were delivered to the primary coil). Both the vagus and the cervical sympathetic trunk were sectioned and then stimulated distal to the section. The saphenous nerve was also stimulated in order to elicit pressor responses.

With adequate stimuli (from 10 to 6 cm. coil separation), we have never seen any indication of the normal vagus inhibitory effect on the heart with slowing in rate and decrease in blood pressure. When the sympathetic trunk was stimulated 2 to 3 cm. below the superior cervical ganglion, no change in blood pressure was observed in 3 experiments with a coil separation of 6 cm. In the

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* This study was conducted with the aid of the Rockefeller Foundation Grant for Fluid Research in the Medical Sciences at Stanford University.

remaining experiments, a rise in blood pressure (1 to 7 mm.) was seen when the sympathetic trunk was stimulated in this position. The latent period was relatively long (2 to 3 sec.). The heart and respiratory rate changed but little and then with slight increases. This effect was present after removal of the superior cervical ganglion at the time of the original operation and thus was not due to descending conduction over axones arising in that ganglion. A similar response was seen only once when the right vagus was stimulated in a similar position and consisted of a 9 mm. rise in blood pressure, an increase of 12 beats per minute in the heart rate and an increase from 37 to 45 respirations per minute. When the electrodes were moved down to about 2 cm. above the middle cervical sympathetic ganglion, the effect could be much exaggerated. Inasmuch as this effect could also be obtained from the vagus with strong stimulation (6 cm. coil separation) at a corresponding level and it resembled the pressor effect obtained from the saphenous nerve, the conclusion was reached that we were dealing with a pressor response elicited probably by the stimulation of sensory fibers either at the point of stimulation or by a spread to ones in the distribution of the middle cervical sympathetic ganglion or possibly in the carotid plexus. The slight increase in blood pressure obtained in some experiments when the sympathetic trunk was stimulated more rostrally may have been due to the occasional presence of a very few visceral afferent fibers which have passed up the cervical sympathetic trunk beyond the middle cervical ganglion. Cleveland's[1] anatomical observations suggest such a possibility. An atypical distribution of visceral afferent fibers probably explains the response which was obtained from the vagus in one instance. This response was present after atropinization (1.2 mg. atropine sulphate intravenously) and disappeared after a dose of fluid extract of ergot which abolished the pressor response from the saphenous nerve. It was also present on stimulation of the peripheral end of the cut normal vago-sympathetic trunk following atropinization.

The effect on respiration as shown by our method of recording was slight and might be attributed as a component of the pressor response. While our observations were not directed especially to the gastrointestinal tract and took place late in the progress of the experiments, no changes in gastrointestinal motility were seen.

Anatomical controls were made by careful autopsy and microscopic sections of the nodose ganglia showed no pathologic abnormalities with the exception of some neuronophagia at the rostral pole of one ganglion.

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[1] Cleveland, D. A., *J. Comp. Neurol.*, 1932, **54**, 35.

Recently Morgan and Goland[2] have presented evidence that tends to show that there are postganglionic parasympathetic fibers which arise in the nodose ganglion of the dog to pass to the heart. Heinbecker and O'Leary[3] failed to show any decrease in heart rate but normal results were obtained from the fibers responsible for certain motor effects in the lungs and duodenum of the cat. Ranson, Foley and Alpert[4] report observations which show no histological evidence that the nodose ganglion contains synapses between pre- and postganglionic parasympathetic neurons in the cat. Our observations in the cat give no support to the presence of postganglionic parasympathetic neurons situated in the nodose ganglion which control the heart. Final conclusions as to the presence of such neurons which may exert an effect on the respiratory and gastrointestinal tracts can not be reached from our observations.

6827

Acetylation Studies.

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In an earlier paper[1] experiments were recorded as evidence for the belief that acetylation studies might prove of value in elucidating phases of intermediary metabolism. In this communication we record further experiments dealing with acetylation.

p-Aminobenzoic acid is partially acetylated by the rabbit forming *p*-acetyl-aminobenzoic acid, a substance which can be recovered from the urine. (Qualitative tests make it quite evident that some of the *p*-aminobenzoic acid is also eliminated in the form of its glycuronate salt; but this will be the subject of another communication.) Since carbohydrates—and possibly fats—are probably the source of the acetyl group furnished for the acetylation process, it became of interest to determine the effect of the injection of insulin upon the amount of acetylated product produced.

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[2] Morgan, L. O., and Goland, P. P., *Am. J. Physiol.*, 1932, **101**, 274.

[3] Heinbecker, P., and O'Leary, J., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 506.

[4] Ranson, S. W., Foley, J. O., and Alpert, C. D., *Anat. Rec.*, 1933, **55** (suppl.), 33.

[1] Harrow, Power and Sherwin, PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 422.

The results show that the injection of 0.5 units of insulin per kilo wt. raised the normal acetylation value of 20-25% to that of 44-47% of the original *p*-aminobenzoic acid injected. Although the rabbits showed individual variations in their ability to acetylate, insulin, in each case, practically doubled the output of the acetylated product.

We next determined what effect the simultaneous injection of insulin and reduced glutathione would have upon the output of the acetylated product. The belief is prevalent that the -SH group has an inhibiting action in so far as insulin activity is concerned. For example, Du Vigneaud[2] has shown that insulin can be inactivated if it is mixed with cysteine or reduced glutathione in a neutral solution in an atmosphere of nitrogen, because the blood-sugar of the rabbit does not drop when the insulin-glutathione (or insulin-cysteine) solution is injected. Instead of mixing our reduced glutathione and insulin *in vitro,* we decided to inject them separately, but simultaneously. Five mg. of reduced glutathione and 0.5 units of insulin (per kilo of body weight) were injected. The results showed an acetylation value of 23-34%, as compared with 44-47% when insulin alone was used.* These *in vivo* experiments confirm Du Vigneaud's *in vitro* results in tending to show that reduced glutathione inhibits insulin action.

Glutathione itself had no effect upon the amount of acetylated product produced. We must, therefore, regard the action of glutathione as being confined to its action on insulin.

The results point to the following conclusions: insulin markedly increases the acetylation process in the body; reduced glutathione does not affect it; but the combined action of insulin and glutathione inhibits the increased output of *p*-acetyl-aminobenzoic acid obtained when insulin alone is used. This supports the view that the -SH group has an inactivating effect upon insulin action.

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[2] Du Vigneaud, *J. Biol. Chem.,* 1931, **94**, 233.

* We are indebted to Professor Du Vigneaud for certain details of his experiments.

6828

Meteorological Reflections in Blood Pressure Rhythm.

WM. F. PETERSEN.

From the Department of Pathology and Bacteriology, University of Illinois College of Medicine.

When daily blood pressure reactions of normal persons or patients ill from a variety of diseases are studied, distinct periodic fluctuations can readily be determined. These periods reveal a distinct association with the meteorological status of the time. Depending principally on the cyclonic circulation of the atmosphere, increase in systolic blood pressure is normally associated with increase in barometric pressure (cold front). This period of relative arteriolar and capillary constriction is associated with a shortening of the methylene blue disappearance time previously described[1] and represents a phase of relative tissue anoxemia. This is in turn followed by arteriole and capillary dilatation as tissue stimulation becomes apparent. During this phase the diastolic blood pressure

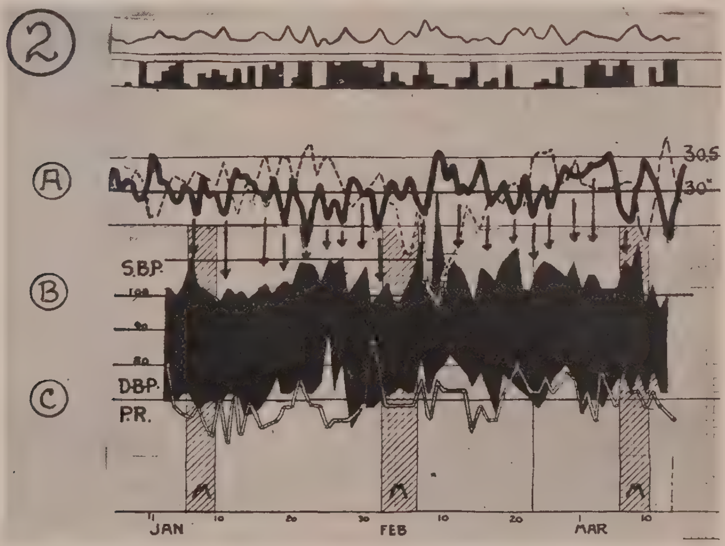

Fig. 1.

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[1] Petersen, Wm. F., and Appelman, B., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 712.

diminishes. Obviously the external temperature would play a rôle in this mechanism.

The appended graph illustrates the close coincidence with alterations in the barometric pressure. The pressor determinations were made in a young woman whose only abnormality is a moderate albuminuria. Studies were made that cover 3 menstrual periods (January 1 to March 13) and the menstrual periods are indicated by 3 cross hatched areas of the chart and marked M. A is the barograph (heavy line). Temperature is indicated by the dotted line. B indicates the systolic blood pressure, and C indicates the diastolic blood pressure. The pulse rate is indicated by the double white line. Arrows have been subtended from low barometric levels to the pressure curves below.

It will be noted that each barometric decline is associated with a systolic pressure increase, the only exception being that of the unusually high pressure recorded on February 9 (unusual cold and psychic effect of a major scholastic examination).

In addition to the meteorological effects which are obvious, the pressor level is of course influenced by the endocrine tide of the sex rhythm. This becomes apparent in this individual in the premenstrual diminution of the diastolic level (January 28 and March 1) and reflects the premenstrual period of stimulation.

6829

Oxidation-Reduction Studies on the 2, 3-Butylene Glycol-Acetyl-Methyl-Carbinol System in a Fermentation.

M. C. BROCKMANN AND C. H. WERKMAN.

From the Department of Bacteriology, Iowa State College, Ames, Iowa.

Dissimilation of glucose is brought about by bacteria of the genus *Aerobacter* and among the products of fermentation are acetyl-methyl-carbinol and its reduction product, 2, 3-butylene glycol. It has been suggested that these substances form a reversible oxidation-reduction system. In this case the methylene blue may replace the acetyl-methyl-carbinol as a hydrogen acceptor providing its redox potential lies above that of the carbinol-glycol system. The substances acting as hydrogen donators include the reduced constituents of all the reversible systems present in fermentations whose electrode values lie below that of methylene blue. In 2, 3-butylene glycol fermentations, lactic acid might be expected to donate hydrogen and form pyruvic acid. If 2, 3-butylene glycol is involved in a reversible system whose electrode potential value is below that of methylene blue, the addition of methylene blue to the fermenting mixture will reduce the concentration of 2, 3-butylene glycol and increase concentration of acetyl-methyl-carbinol.

The medium used was 0.2 molar glucose solution containing 1 gm. of ammonium chloride and 1 gm. of dipotassium phosphate per liter. The pH was held constant by the addition of 10 gm. of calcium carbonate to each liter of fermenting medium. The fermentation took place under anaerobic conditions produced by a continuous stream of CO_2 bubbling through the medium. The organisms used were *Aerobacter indologenes*, *A. salicinovorum* and *A. decolorans*.

When ⅔ of the glucose was fermented, a known quantity of methylene blue was added to the fermentation. Samples were withdrawn after the addition of various amounts of methylene blue and the 2, 3-butylene glycol, acetyl-methyl-carbinol and glucose were determined. The results of these analyses are shown in Table I.

TABLE I.
Fermentation of Glucose by *Aerobacter salicinovorum*.

Time, hr.	Molarity Glucose[1]	Molarity Glucose (detd)	Moles Glucose Used[2]	Molarity Acetyl-methyl-carbinol	% Yield Acetyl-methyl-carbinol (molar basis)	Molarity 2, 3-Butylene Glycol	% Yield 2, 3-Butylene Glycol	Normality Methylene Blue	cc. 1N Methylene Blue per cc. 1M Glucose Used
0	.195	.195	.000	——	—	——	——	——	——
93¾	.193	.063	.130	.0015	1.2	——	——	.0014	——
94¾	.193	.061	.132	.0012	0.9	——	——	.0014	——
95¾	.193	.060	.133	.0004	0.3	.0781	58.4	.0014	.0105
103¼	.191	.041	.150	.0022	1.5	——	——	.0026	.0173
116¼	.190	.007	.183	.0025	1.2	.1009	55.1	.0037	.0202
144¾	.180	——	.180	.0017	1.0	——	——	.0118	.0655
244	.155	——	.155	.00185	1.2	.0869	56.3	.0323	.2084
312	.139	——	.139	.0019	1.4	.0808	56.4	.0438	.3151

[1] Corrected for dilution due to addition of methylene blue solution.
[2] Difference, columns 2 and 3.

The results seem to show that the 2, 3-butylene glycol does not act as a hydrogen donator to methylene blue under the conditions of this experiment and hence cannot be included in a reversible system.

However, it may be that the electrode potential for the 2, 3-butylene glycol-acetyl-methyl-carbinol system lies above that of the methylene blue-methylene white system. At the pH of these experiments the electrode potential of this methylene blue-methylene white system is 0.039 volts. There is a possibility that this potential might lie below that of the 2, 3-butylene glycol-acetyl-methyl-carbinol system. Consequently, this experiment was repeated substituting for the methylene blue, o-chlorolphenol-indophenol, the electrode value of which is 0.288 volts. The results of this experiment confirmed the results obtained with methylene blue.

It appears, therefore, under the conditions of our experiments that 2, 3-butylene glycol and acetyl-methyl-carbinol do not constitute a reversible oxidation-reduction system.

6830

Differentiation of Gonad Primordia in Kidney of Adult Rat.

ADRIAN BUYSE. (Introduced by R. K. Burns, Jr.)

From the Department of Anatomy, School of Medicine and Dentistry, University of Rochester.

Transplantation of mammalian embryonic tissues to an adult environment has been more or less successful in the hands of several workers. Sites within the abdomen of the rat have not proven very successful as reported by Nicholas.[1] Nicholas has, however, obtained differentiation in 80% of grafts of 8 and 9 day rat embryos transplanted to the adult mammary gland. In the rabbit, Waterman[2] has demonstrated that the omentum of an adult animal serves as a suitable site for the differentiation of embryonic primordia.

In the present work various abdominal sites were tried with poor success. The kidney, however, as a site for the transplantation of embryonic primordia offers a number of advantages. Growth is unrestricted, the capsule interfering little with the graft, which is clearly visible and sharply differentiated from the host tissue. Rapid vascularization insures ready incorporation of the transplanted tissue. These advantages are demonstrated by the transplantation, in a subcapsular position, of a series of grafts of posterior halves of 11 day rat embryos, or the mesonephroi and gonad primordia of 12, 13 and 14 day embryos (sex is not morphologically differentiated until the fourteenth day), for periods of 7 to 28 days. In one series of 116 grafts, 80% of the grafts have become established. The number of grafts differentiating in a second series of transplantations of 64 cases has been 100%. Fourteen to 21 days is the period of optimum differentiation.

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[1] Nicholas, J. S., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 188.

[2] Waterman, A. J., *Am. J. Anat.*, 1932, **50**, 451.

From posterior halves of 11 day embryos skin, nervous tissue, connective tissue, cartilage, bone, smooth and striated muscle, metanephros, gonad, adrenals and gut differentiate. When the mesonephroi and gonad primordia are transplanted alone there is subsequent development of metanephros and sexually differentiated gonad.

When morphologically indifferent gonad rudiments are implanted on the kidneys of adult male rats, they differentiate in the presence of the adult male sex hormone. Typically normal male gonads differentiate even to the establishment of rete tubules and vasa efferentia. The results are as follows: (1) Many more testes differentiate than ovaries, 60% of all cases. (2) Only 17% of the gonads differentiating are ovaries. Although very small, these are sometimes fairly normal, with developing follicles containing young growing oögonia and oöcytes. (3) 9.5% of the gonads differentiating in male hosts exhibit a bisexual structure. These gonads contain both medullary and cortical components. (4) Sex cannot be identified in 12% of the gonads differentiating. In these indeterminate grafts, sex cords are irregularly arranged adjacent to or in connection with the rete ducts.

A number of grafts classed as small testes should be considered as transformed ovaries. These cases exhibit medullary cords transforming into seminiferous tubules. This interpretation is also supported statistically. Of a series of 116 grafts in mature male hosts, 70 are testes. If all of the bisexual gonads, the sexually indeterminate grafts, and ovaries, be classed as zygotically determined females the number is but 46, and is still overbalanced by a preponderance of males. With the 70 testes have been classed a group of 9 small gonads which give evidence of a transformation from the female condition. If these be added to the female side of the series the ratio is changed to 61 males and 55 females, a ratio approximating the normal 50 to 50 ratio.

The results at first suggest inhibition, or a partial or complete reversal, of some female primordia through the action of a hormone; but this interpretation, though in agreement with the freemartin theory, must be abandoned after examination of a series of 64 grafts resident in mature female hosts. These gonads give approximately the same ratio as obtained in the male hosts, eliminating the possibility of explaining the results on the basis of a hormone action. Only one acceptable explanation can be advanced, namely that the developing gonad has the ability to differentiate according to its żygotic determination, even in the presence of adult sex hormones. This self-differentiating potency is not, however, equal for the medullary and cortical components. The medulla of the differentiating gonad has a greater capacity for self-differentiation in the experimental environment than the cortical area. Willier[3] by transplanting undifferentiated gonad primordia of chick embryos to the chorio-allantoic membrane of host embryos has observed self-differentiation of the gonad grafts independent of the hormones of the host.

6831

Excretion of Estrin in Acne.

THEODORE ROSENTHAL AND RAPHAEL KURZROK.
(Introduced by J. G. Hopkins.)

From the Departments of Dermatology, and Biochemistry and Obstetrics and Gynecology, Columbia University.

On clinical grounds it has long been suspected that acne vulgaris is caused by some endocrine disturbance—presumably by disturbance of the gonads (Pick,[1] Hollander,[2] Schamberg,[3] Darier,[4] Bloch,[5] Stein,[6] and others). The appearance of acne usually coincides with the endocrine changes for the sexual maturity associated with puberty. Only in exceptional instances is there any evidence that these changes are abnormal as for example, the case cited by Bloch in which acne appeared in the first years of life when (owing to suprarenal tumor) sexual maturity was precociously developed.

It seemed to the writers that evidence of normality would be tested by a study of the excretion of estrin and prolan in women with acne. Previous studies[7] have shown that normal women between the ages of sex maturity and the menopause excrete from 10 to 20 rat units of estrin per liter of urine throughout the menstrual cycle. Prolan is not found by the methods here employed in such cases.

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[3] Willier, B. H., *J. Exp. Zool.*, 1927, **46**, 409.

[1] Pick, *Arch. f. Dermat.*, 1921, **131**, 350.

[2] Hollander, *Arch. Derm. and Syph.*, 1921, **3**, 593.

[3] Schamberg, *Arch. Derm. and Syph.*, 1921, **4**, 293.

[4] Darier, *Precis de Dermatologie*, Masson et Cie, 1928, 508.

[5] Bloch, *Brit. J. Derm.*, 1932, **43**, 61.

[6] Stein, *Handbuch der Hautkrank.*, 1932, **13**, 81.

The urines of 34 young women who applied to Vanderbilt Clinic for treatment of acne were examined. The age range was 11 to 33 years, 21 being under 20 years of age. Estrin was determined by the method of Kurzrok and Ratner,[8] and prolan by the method of Zondek.[9]

Ovarian hormone was completely absent in 27 of these cases, present in normal amounts (10-20 rat units) in 6 cases, and slightly positive in one case (4 rat units).

Work has not progressed to a stage from which final conclusions can be drawn, and the results of treatment with preparations of ovarian follicular hormone and anterior pituitary prohormone will be reported later, but a number of patients who formerly excreted no hormone were found to be excreting hormone after treatment.

The consensus of opinion of those who have studied the question is that oestrin is present with considerable regularity in the urine of normal women throughout their menstrual life. Three individuals studied with frequently repeated tests gave the following results:

Normal No. 1, age 27, six days out of one week all specimens showed oestrin varying from 3 to 6 R.U.

Normal No. 2, age 35, examined on 33 days out of 7½ consecutive weeks, 30 specimens were positive showing from 3 to 18 R.U. of oestrin. Three specimens were negative.

Normal No. 3, age 30, examined 28 days out of 30, 18 specimens were positive showing from 4 to 9 R.U. Two specimens showed less than 9 R.U. and 8 specimens were negative.

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[7] Kurzrok, *N. Y. State J. Med.*, 1932, **32**, 1287.

[8] Kurzrok and Ratner, *Am. J. Obs. and Gyn.*, 1932, **23**, 689.

[9] Zondek, *Z. Gynak.*, 1929, **53**, 1, 834.

6832

Endocrine Reactions in Female Rats after X-Ray Treatment of the Ovaries.*

W. T. LEVINE AND EMIL WITSCHI.

From the Zoological Laboratory, The State University of Iowa.

X-ray sterilized rats with completely destroyed germ cells but well preserved somatic elements of the testis, fully maintain their secondary sex characters.[1] They react like total castrates when united in parabiosis with untreated females. This was taken as evidence that the X-ray sterilized male, like the castrate, produces large amounts of follicle stimulating hormone of the anterior pituitary. So-called castrate cells are found in the hypophyses of X-rayed males, though in smaller numbers than in surgical castrates. Parkes[2] first observed that X-ray sterilized female mice in many instances maintain fully their secondary sex characters including the cyclical oestrus changes. Ford and Drips[3] later reported experiments on rats which led to similar general conclusions. We endeavored to correlate the ovarial changes with the hypophyseal reactions. The main results and conclusions can be summarized as follows:

According to the extent of damage of ovarial structures observed at 4 to 7 months after irradiation the females can be divided into 2 groups. In both the germ cells have completely disappeared but the follicular apparatus—cystic follicles, corpora lutea—is partly preserved in the ovaries of the first group and completely destroyed in those of the second group. Ovaries of the second group show a profuse proliferation of epithelial cells from the germinal epithelium which never become organized into follicular structures. In addition, small "anovular follicles" may arise from the same source, though they soon disintegrate without ever exhibiting cystic transformations.

Females of Group I, 12 cases, observed over periods up to 7 months after irradiation, maintained fairly well the cyclical oestral changes observed in vaginal smears. Periods of 3 to 6 cycles of normal lengths usually were alternating with periods of prolonged

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* Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council.

[1] Witschi, E., Levine, W. T., and Hill, R. T., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1024.

[2] Parkes, A. S., *Proc. Roy. Soc.*, B., 1926, **100**, 101.

[3] Ford, F., and Drips, D., *Radiology*, 1929, **12**, 393.

oestrus or, less frequently, by prolonged dioestrus. If killed during the oestral period the uteri were found distended, though not fully to the size of normal controls. The histological picture of the hypophyses is essentially normal. Untreated females in parabiosis with females of this group stay in constant oestrus for periods of several months.

Females of Group II, 17 cases, according to their vaginal smears either stay in constant oestrus or in constant dioestrus (observed duration 2 to 5 months). The uteri are slightly stimulated in the first case and of the castrate type in the second case. In the anterior lobe of the hypophyses large vesicular castrate cells are present in varying numbers. Parabiotic untreated females stay in constant oestrus.

The untreated parabiont in constant oestrus always possesses a histologically normal hypophysis. Her ovaries are crowded with cystic follicles of mature size or slightly larger. Corpora lutea are entirely absent. Evidently only the follicular growth stimulating hormone of the sterilized twin is carried over in quantities surpassing the threshold value. Since this reaction appears already in Group I it is obvious that castrate cells are not necessarily responsible for the excess production of the follicle stimulating hormone.

The fact that cyclical oestrus changes are observed only as long as parts of the follicular apparatus are retained does not necessarily prove that the rhythmical phenomena depend on these morphological formations. Parkes'[2] observations on mice rather seem to suggest that our 2 groups are only differing in a quantitative way.

While the ovary deprived of all germ cells still is able to produce hormones controlling secondary sex characters and even to maintain cyclical phenomena, it appears that in all cases the output is quantitatively reduced.

6833

Further Studies on Copper and Iron.

H. L. KEIL, H. H. KEIL AND VICTOR E. NELSON.

From the Laboratories of Physiological Chemistry, Iowa State College, Ames.

Waddell, Steenbock, Elvehjem, and Hart[1] showed that rats fed milk exclusively developed nutritional anemia and that the ingestion of pure iron salts failed to cure it. However, normal regeneration of hemoglobin was obtained with small additions of copper sulphate along with the iron salt. These results have been confirmed by Keil and Nelson.[2] Myers and Beard[3] found that higher doses of zinc and magnesium retarded blood regeneration. If this is the case, anemia should develop more rapidly on milk and $FeCl_3$ if these elements are included. The slope of the curves obtained with and without zinc or magnesium demonstrate that anemia resulted as readily with or without these elements.

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[1] Hart, E. B., Steenbock, H., Elvehjem, C. A., and Waddell, J., *J. Biol. Chem.*, 1928, **77**, 777.

Prior to this work on hemoglobin regeneration, Daniels and Hutton[4] demonstrated that rats failed to reproduce on milk and iron, but reproduction was a success when a mineral mixture of NaI, NaF, $KAl(SO_4)_2$, $MnSO_4$, and Na_2SiO_3 was added. They concluded that all of these salts were necessary in the milk for reproduction. The work of Hart, Steenbock and associates[1] and Keil and Nelson[2] is so conclusive, that it is certain animals cannot grow and reproduce normally without copper. There must have been contamination of this element either in the milk or salt mixture used by Daniels and Hutton. The minimum amount of copper required for regeneration of hemoglobin is extremely small. The minimum amount of copper as $CuSO_4$, when injected intraperitoneally, is shown to be 0.002 mg. daily. The milk used in these experiments was collected directly into glass containers in order to avoid contamination with copper. Milk *ad lib* and 0.5 mg. of Fe as $FeCl_3$ daily were fed to the rats. 0.05 mg. of Fe as $FeCl_3$ injected intraperitoneally with 0.002 mg. Cu as $CuSO_4$ daily does not result in normal regeneration. When the iron as $FeCl_3$ was increased to 0.1 mg. daily there was further stimulation of hemoglobin formation; but regeneration was not normal as shown by the plateau of the hemoglobin curve. At this point 0.15 mg. of colloidal Fe was administered intraperitoneally daily, and as a result regeneration to the normal level of hemoglobin occurred. Injections intraperitoneally of 0.05 mg. and 0.1 mg. of Fe as $FeCl_3$ caused necrosis and sloughing off of the skin at the point of application; hence colloidal Fe was used. The colloidal Fe was free from dialyzable Fe.

Rats on milk collected in glass together with $FeCl_3$ and $CuSO_4$ grew at a rate considerably slower than rats on our stock ration. The addition of Na_2SiO_3 to the above ration did not improve the growth curves. However, $KAl(SO_4)_2$ in place of the silicate gave better results than the other 2 rations. Sodium fluoride when added to the milk, Cu and Fe ration gave results which surpassed those on $KAl(SO_4)_2$ for the first 4 months; then the fluoride exhibited toxicity. Rats on milk, $FeCl_3$, and $CuSO_4$ and the Daniels and Hutton salt mixture, exclusive of NaI, grew satisfactorily and performed equally well as rats on our stock ration. Rats on milk, $FeCl_3$, $CuSO_4$, and $MnSO_4$ performed better than any of the previous lots and even surpassed those on the stock ration. Reproduction on milk, $FeCl_3$, $CuSO_4$, and the Daniels and Hutton salt mixture was very good, during 3 generations studied. The rats on milk, copper, and iron in the first generation reproduced equally well with the animals on the Daniels and Hutton salts, but the mortality of the young was considerably greater in the former case. Furthermore, the weight of the young at birth and weaning time was considerably less in the former case than on the Daniels and Hutton salts. Male and female rats of the first generation on milk, copper, and iron proved fertile for at least 20 months and are still active in this respect. However, rats on this same diet do not reproduce in the second generation. The animals of the second generation failed to reproduce in 8 months; but with the addition of the Daniels and Hutton salt mixture reproduction occurred, and the per cent mortality of the young was low. Infection of the middle ear, *otitis media*, was very prevalent in the second generation rats receiving milk, iron, and copper; but it was not nearly so common in the first generation. Only 2 of the 88 animals on milk, Cu, Fe, and Mn as $MnSO_4$ showed this pathological condition. 6 to 12 rats were employed in each experiment.

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[2] Keil, H. L., and Nelson, V. E., *J. Biol. Chem.*, 1932, **97**, 115.

[3] Myers, V. C., and Beard, H. H., *J. Biol. Chem.*, 1931, **94**, 89.

[4] Daniels, A. L., and Hutton, M. K., *J. Biol. Chem.*, 1925, **63**, 143.

6834

Cardiovascular System in Acute Experimental Peritonitis.

BERNHARD STEINBERG, J. LESTER KOBACKER AND TOM G. RUSSELL.

From the Department for Medical Research, Toledo Hospital, Toledo, Ohio.

The purpose of this work is to determine the reaction of the cardiovascular system to experimental peritonitis. Holzbach[1] in his review on circulatory failure in infectious conditions states that functional heart failure appears early in peritonitis, but this opinion is apparently based on clinical observations since no experimental work of this condition is reviewed.

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[1] Holzbach, E., *Wurzb. Abhandl. a. d. Gesamtgeb. d. Med.*, 1931, **27**, 1.

Two types of peritonitis were induced in dogs. Fecal peritonitis was produced in 5 dogs by the intraperitoneal introduction of 5 gm. of small and large bowel feces suspended in 25 cc. of saline. Colon bacillus peritonitis was induced in another set of 5 dogs by the intraperitoneal injection of 50 cc. of a 2% gum tragacanth in saline containing 200 million *B. coli* per cc. In each of the animals blood pressure was obtained prior to and continuously after the onset of peritonitis by insertion into the carotid artery of a cannula which was connected to a mercury manometer and recorded on a kymograph. The animals were under pento-barbital sodium anesthesia. With each animal with peritonitis a normal control dog was run simultaneously. In another set of 4 dogs, 2 with fecal peritonitis and 2 with *B. coli* peritonitis, electrocardiograms were taken prior to and at intervals after the onset of peritonitis.

There was a gradual drop of blood pressure beginning within 15 to 20 minutes after the onset of peritonitis. In one hour the carotid pressure dropped 2 mm. of Hg. The pulse pressure became progressively smaller. The fall was of the systolic pressure, the diastolic being maintained. In 6 hours the carotid pressure dropped 5 mm. of Hg. and an arhythmia appeared. In *B. coli* peritonitis the carotid pressure drop was more rapid but seldom exceeded 4 mm. of Hg. The electrocardiographic study revealed an increase in pulse rate (from 90 to 220 in 4 hours) with an auriculo-ventricular response of 1:1. The ST interval was depressed and the T wave was diphasic. In 6 hours the pulse rate had further increased (to 264) and there was a greater T wave deformity. The changes in the electrocardiograms in fecal and *B. coli* peritonitis were identical and indicated myocardial damage. Histological sections of the peritoneum revealed dilated capillaries engorged with blood. This marked dilatation and engorgement became apparent within one hour after the onset of the infection.

In a former article one of us (B.S.)[2] demonstrated that the cause of death in peritonitis is due to liberation of soluble bacterial toxic substances within the peritoneum. With the observation of dilated and engorged peritoneal capillaries, the cause of the cardiovascular collapse is suggested to be due both to the toxic action of the soluble toxins on the myocardium and a local peritoneal stagnation of blood.

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[2] Steinberg, B., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 59.

6835

Effect of Irradiating Non-Goitrogenic Cabbage on its Goitrogenic Activity.

J. J. WESTRA* AND VIOLA HUNTER. (Introduced by A. J. Carlson.)

From the Department of Physiology, University of Chicago.

The effect of irradiation on the goitrogenic activity of cabbage was recently reported by Webster.[1] The cabbage employed by Webster possessed some goitrogenic activity before irradiation.

The experiment here reported were undertaken to see whether prolonged irradiation of a non-goitrogenic variety of cabbage would make it goitrogenic. All the cabbage used was obtained from the same source. It was finely hashed, spread out in layers 1 to 2 cm. thick, and irradiated for 30, 60, or 120 minutes at a distance of 50 cm. with a Cooper-Hewitt mercury vapor lamp, ultra-violet limits 2252-2224 A.U. One week before cabbage feeding was begun the thyroid glands of all animals were examined under ether anesthesia, and each animal was injected subcutaneously with 5 mg. KI to produce uniform involution of the thyroid. The cabbage was fed in

TABLE I.

Group	No.	Body Wt. gm.	Thyroid Wt. mg.	Days on Diet	Av. Thyroid Wt.-mg.	Thyroid Wt./kg. Body Wt.-mg.
A Normal Diet	1	2890	261	—	201	86
	2	2285	135	—		
	3	1925	207	—		
B Unirradiated Cabbage	1	1860	90	126	93	75
	2	1380	80	91		
	3	1975	110	126		
C—Cab. Irrad. 30 min.	1	2970	205	126	181	75
	2	2085	170	126		
	3	2115	172	126		
D—Cab. Irrad. 60 min.	1	2025	135	126	128	68
	2	1790	121	28		
	3	1860	130	126		
E—Cab. Irrad. 120 min.	1	1900	120	126	110	51
	2	2175	110	126		
	3	2460	100	126		

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* This work was aided by a grant from the Henry Strong Denison Medical Foundation.

[1] Webster, B., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1070.

quantities equivalent to 60 calories kg. per day. This constituted the only food for the first 12 weeks. In the last 6 weeks of the experiment 50 gm. of oats were given to each animal once a week. After 18 weeks of cabbage feeding, the animals were killed, and the thyroid glands were weighed.

The results (Table I) indicate that the prolonged feeding of cabbage which is originally not goitrogenic, and is irradiated in the manner described does not produce thyroid enlargement. Nongoitrogenic cabbage, therefore, does not seem to contain the precursors of a goitrogenic substance that can be synthesized by prolonged irradiation under the conditions stated for this experiment.

6836

Vestibular Function in Experimental Beriberi.

CHARLES F. CHURCH. (Introduced by D. W. Bronk.)

From the Johnson Foundation for Medical Physics and Department of Pediatrics, University of Pennsylvania.

The neuromuscular manifestations of beriberi (vitamin B_1 deficiency), the so-called "convulsions, spasticity and paralysis", simulate in some respects the disturbances of equilibrium and coordination which follow injury to the labyrinths. It has been suggested that these manifestations are the consequence of a lesion of the vestibular apparatus, but histological examination has failed to substantiate this hypothesis.[1] The investigation here reported was therefore devoted to a functional examination of the vestibular system to gain further information regarding the existence of such a lesion.

Vestibular function was tested by a modification of the well known Barany test. The character and duration of nystagmus following a standardized rotational stimulus served as the index. More than 2,000 tests have been made on 84 rats under various experimental conditions. The basal diet used throughout these experiments, when supplemented with codliver oil and brewer's yeast, permitted normal growth and health. When the whole yeast in the supplement was replaced by autoclaved yeast, however, the neuromuscular manifestations of beriberi appeared in 5 to 7 weeks.

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[1] Woollard, H. H., *Australian J. Exp. Biol. and Med. Sci.*, 1932, **9**, 173.

The standard rotational stimulus was given by means of an electric phonograph motor and turntable upon which the animal was placed with its head held in the normal resting position at the center of the rotating disk. A speed of 2 revolutions per second was employed and this speed was attained in approximately 8 seconds. At the end of 20 seconds' rotation the electromagnetic brake was applied, bringing the turntable to a stop in 3 to 4 seconds. With the aid of a metronome the seconds were counted off from the moment the brake was applied until the pendular movements of the eyes induced by rotation had ceased. The animal was completely covered during rotation. A definite relation between speed of rotation and duration of nystagmus has been worked out. The time of rotation was such as to produce the maximal response in normal animals. The vestibular test consisted of clockwise rotation and observation of the duration of nystagmus followed immediately by counterclockwise rotation and observation. If the results did not agree within 3 seconds the observations were repeated and averaged with the first. The vestibular tests were performed on beriberi and control animals of the same litter in immediate sequence and were repeated several times a week. Many of the beriberi animals were treated after the appearance of neuromuscular symptoms and the tests were continued during recovery. Some received a single dose of antineuritic vitamin sufficient to allay the acute symptoms and were followed to subsequent recurrence.

The results are as follows: 1. The duration of nystagmus induced by a standard rotational stimulus in young normal rats varies but slightly from day to day. It diminishes somewhat with repetition and with approach to maturity. These results are in agreement with the work of Detlefsen.[2] 2. The duration of nystagmus in beriberi rats after a 4 week depletion period is consistently greater than in the controls, increasing as the animal becomes more deficient, with a rapid rise to the maximum (which may be 2 to 3 times the normal) 2 or 3 days before the appearance of the usual neuromuscular symptoms. 3. During the acute stage of beriberi the nystagmus following rotation may be (a) prolonged as in 2 above, (b) atypical in character (*e. g.,* rapid nystagmoid oscillations gradually dying away), or (c) entirely absent. Spontaneous nystagmus is sometimes seen. Although complete loss of nystagmus does not always occur during the first acute attack it is usually associated with recurrence of the neuromuscular symptoms in protracted deficiency.

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[2] **Detlefsen, J. A., *Proc. Am. Phil. Soc.*, 1923, 62, 292.**

4. The vestibular response of the beriberi rat shows a rapid change toward normal following treatment with brewer's yeast or heat-labile fraction. After complete loss of nystagmus the characteristic response appears as early as 4 hours following treatment, although it is still prolonged. When treatment is continued daily the duration of the response drops to normal limits within a week. 5. The increased duration of the vestibular response, together with certain qualitative changes, appears to be characteristic of deficiency of the heat-labile fraction of vitamin B. All other factors so far investigated, including partial and complete starvation, middle and inner ear infections ("mastoid disease" of Detlefsen[2]), vitamin A deficiency, and vitamin G deficiency, either diminish the duration of the response or are without effect. 6. These results indicate an early involvement in beriberi of the vestibular system or of the nerve pathways serving vestibular nystagmus.

6837

An Easily Inserted Flexible Cannula.

HENRY R. JACOBS. (Introduced by A. Baird Hastings.)

From the Department of Medicine, the University of Chicago.

In the development of a technique for constant intravenous injection a cannula was devised which is easily manipulated and which has proved useful for the injection and removal of fluids over extended periods of time. It is introduced into a vein through a needle, after the manner of a trocar and cannula, and, when in place, provides a flexible, elastic, and resilient channel for the passage of liquid. It has been found satisfactory not only for intravenous work but also—by Dr. Henry N. Harkins of our Department of Surgery—for experimentation with cerebrospinal fluid through cisterna puncture. No doubt it will prove to be a useful instrument for the cannulation of many of the spaces in the body.

The cannula is made of high grade anode rubber in the form of a capillary tube. Although there is no theoretical limit to the size of a cannula which may be used in this manner, one with an outside diameter of 0.04 inch has been found satisfactory. The cannula is inserted in the following way. First, the cannula is "threaded" in a syringe partly filled with injectable liquid (Fig. 1). A knot should be tied in the proximal end of the cannula to prevent its complete escape. Then a Luer needle (Size 15 with this tube) of proper length is introduced into the space to be cannulated. The "threaded" syringe is attached to the needle, and, with a thrust on the plunger, the cannula is ejected through the needle. The syringe is detached, the knot in the tube cut off, and the needle withdrawn. The cannula now lies free in the tissues and connects the space with the exterior. It may be attached to the rest of the apparatus through a fine Luer needle. If the cannula is to remain in place for a long time, it should be fastened to the skin near its point of exit by a strip of adhesive tape.

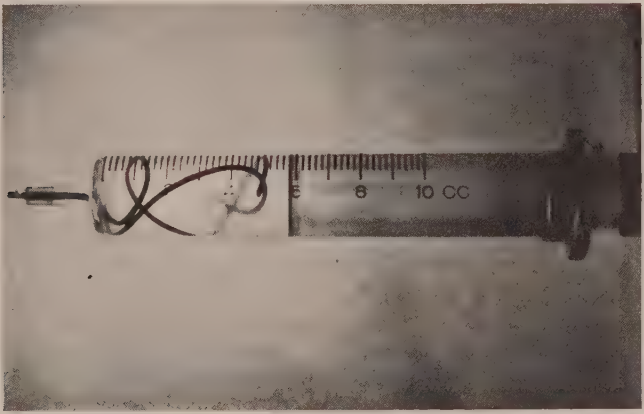

FIG. 1.
A syringe "threaded" with the cannula.

The advantages which this type of cannula offers are that cannulation of many quite inaccessible spaces is made easy and bloodless; that the procedure can be made a sterile one; that the cannula may remain in place indefinitely without discomfort to the subject; and that, because the cannula is flexible, the subject need not be kept motionless during the experiment.

(This cannula can be had from the Oak Mfg. Co., 711 West Lake Street, Chicago, Illinois.)

6838

Preparation of Crystallized Egg Albumin (Ovalbumin).

ARTHUR G. COLE. (Introduced by W. H. Welker.)
From the Department of Physiological Chemistry, University of Illinois College of Medicine, Chicago.

Sörensen[1] indicates that crystallized egg albumin, in contrast to most common proteins, may be considered to be a distinct chemical individual. Since this property makes it especially suitable for studies on the physico-chemical and immunological behavior of proteins, a simple, dependable method for its preparation is desirable.

In all the standard methods for the preparation of crystallized egg albumin, fresh egg white is first treated with an equal volume of saturated ammonium sulphate solution, to precipitate the so-called egg "globulin". The filtrate from this sticky, dough-like precipitate of globulin is then ordinarily brought to the neighborhood of the iso-electric point of the ovalbumin, where crystallization takes place, by the addition of the required amounts of acetic, hydrochloric or sulphuric acids. Hektoen and Cole[2] pointed out that the voluminous precipitate of "globulin" obtained from egg white is not a true globulin, but a mixture or compound of all the proteins in egg white. When extracted with distilled water, the greater portion of it dissolves, leaving behind a clear, gelatinous residue which has the physical properties, at least, of a mucin. The water soluble portion yields crystals of ovalbumin, and contains large quantities of the non-crystallizable conalbumin as well as some ovomucoid.

In the present method, fresh egg white is beaten lightly to break up the membranes, strained through cheese cloth and then acidified by the gradual addition, with constant stirring, of one volume of N/1 acetic acid for each 9 volumes of egg white. Measurements made with the quinhydrone electrode on numerous samples of fresh egg white indicate that this amount of acid is sufficient to bring the material to a pH of 5.0 or less, where, as shown by Sörensen and Höyrup[3] crystallization of the ovalbumin readily takes place. Acetic acid is used in preference to a stronger acid because it may be used in this concentration with less danger of passing beyond the crystallization zone. This acidification of the egg white causes the separation of a comparatively small, gelatinous precipitate (probably ovomucin) which may be removed easily by straining the material through cheese cloth. The filtrate obtained in this manner no longer exhibits the sliminess and viscosity characteristic of egg white, but may be readily filtered through ordinary filter paper. The clear filtrate is then brought to 40% saturation with ammonium sulphate, and filtered from the slight precipitate formed at this point. To the clear filtrate, saturated ammonium sulphate solution is now added, a little at a time, with constant stirring, until a highly opalescent solution is formed. This solution is then permitted to stand at room temperature, with frequent stirring, until crystallization takes place. Crystallization ordinarily begins in a few hours, and within 24 hours a voluminous precipitate of needle-like crystals usually separates out. The crystallization process is permitted to continue for 5 to 7 days, the mixture being stirred occasionally to facilitate the establishment of equilibrium between the crystals and the mother liquor. At the end of this time, the crystals are filtered off and permitted to drain as completely as possible from the mother liquor. They are then washed by covering them, on the filter paper, with a solution of ammonium sulphate which just fails to give a turbidity when treated with a small portion of the mother liquor. After this wash liquid has drained off, the bulk of the precipitate is scraped off the filter paper and transferred to a clean beaker. The filter paper is then extracted several times by maceration with small quantities of distilled water and the extracts, after removal of the filter paper by straining through cheese cloth, are added to the main portion of precipitate. The mixture is then stirred gently, with addition of more distilled water if necessary, until completely dissolved.

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[1] Sörensen, S. P. L., *Compt. rend. trav. Lab. Carlsberg*, 18. No. 5, 1930.

[2] Hektoen, L., and Cole, A. G., *J. Infect. Dis.*, 1928, **42**, 1.

[3] Sörensen, S. P. L., and Höyrup, M., *Compt. rend. trav. Lab. Carlsberg*, 1917, **12**, 213.

For purposes of recrystallization, this solution of ovalbumin is filtered and the clear filtrate treated with a buffered solution of saturated ammonium sulphate. This latter solution is prepared by mixing one volume of acetate buffer (N/1 acetic acid + N/1 sodium acetate) having a pH of approximately 4.7 with 9 volumes of saturated ammonium sulphate solution and then saturating the mixture with solid ammonium sulphate. This buffered ammoniun sulphate solution serves to maintain the ovalbumin, without further manipulation, at the proper pH for crystallization. It is added to the ovalbumin solution, a little at a time, with constant stirring, until a highly opalescent solution is again formed. The procedures for crystallization, washing and re-solution of the crystals are now the same as described above, and the crystallizations may be repeated as many times as desired. Hektoen and Cole,[2] using the precipitin reaction, confirmed Sörensen and Höyrup's[4] observation that at least 3 such crystallizations and washings are required to free the ovalbumin completely from conalbumin. After the final crystallization and washing, the ovalbumin is dissolved in distilled water, placed in collodion or cellophane bags under toluol and dialyzed against distilled water until free from salts.

6839

Meteorological and Menstrual Reflections in Nail Growth.

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When daily measurements of nail growth is recorded in the normal person or in patients, distinct periods of expansion and retraction of the nail can be observed, as well as variations in the rate of growth.

Periods of cold and increasing barometric pressure (cyclonic cold fronts) are associated with cessation of growth and retraction of the nail, periods of increasing temperature and barometric decline with increase in the rate of growth. However, similar fluctuations occur in subjects kept constantly at normal room temperature (68 to 72°F). The periods of increase in the rate of growth apparently reflect periods of general stimulation that follow the anoxemia.

In the graph which illustrates observations made on the same individual described in a preceding note,[1] the actual growth curve of the nail is recorded. Arrows have been carried up from periods of high barometric pressure which, it will be observed, correspond to a time of retraction of the nail or slowing of its rate of growth; expansion or increase in the rate of growth occurs with diminution of the barometric pressure.

Premenstrually an acceleration in the rate of growth is observed and an unusual sensitiveness of the nail to the minor meteorological disturbances of the premenstrual period (February 27-March 6).

The rate of nail growth apparently reflects very accurately the meteorological environmental influences.

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[4] Sörensen, S. P. L., and Höyrup, M., *Compt. rend. trav. Lab. Carlsberg*, 1917, **12**, 12.

[1] Petersen, W. F., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1145.

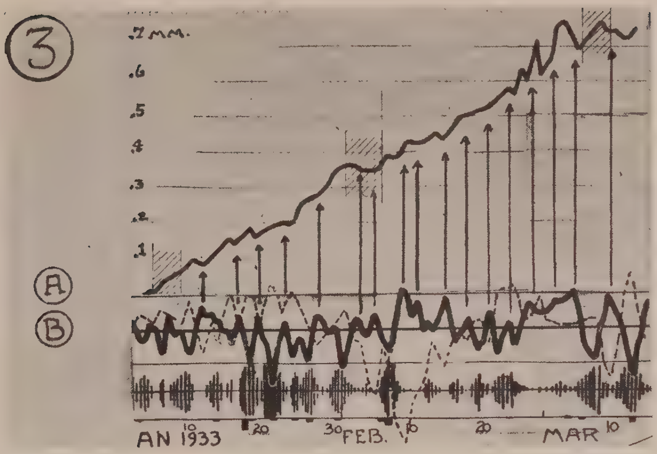

Fig. 1.

On the small chart A represents the nail growth in millimeters, B is the barograph, and menstrual periods have been indicated by cross hatching.

6840

Action of Theelol (Tri-Hydroxy-Oestrin) on Uterine Fistulae in the Unanesthetized Rabbit.

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From the Department of Physiology, Western Reserve University School of Medicine, Cleveland.

The present paper presents observations relating to the effect of theelol (tri-hydroxy-oestrin) on the quiescent non-gravid uterine fistula of the castrated adult rabbit. The method of recording such contractions has been described.[1, 2] The data embody observations following the intravenous and subcutaneous administration of theelol (Parke, Davis and Co.) totalling 19 times in 8 rabbits. The uterine response to theelol was as follows:

Intravenous Administration. Three castrated rabbits with quies-

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[1] Reynolds, S. R. M., *Am. J. Physiol.*, 1930, **92**, 420.

[2] Reynolds, S. R. M., and Friedman, M. H., *Am. J. Physiol.*, 1930, **94**, 696.

cent uteri were treated with theelol as follows: Four divided doses covering 8 hours were given and records of motility obtained before, during, and from 24 to 48 hours following the injections. The amount injected was comparable to the threshold amounts of theelin (keto-hydroxy-oestrin) similarly administered as reported before.[3] In each of the records of the present series, a beginning of rhythmical motility by 10 hours following the first injection occurred and by 24 hours the contractions were of duration, amplitude and frequency which was entirely comparable to and indistinguishable from those which follow intravenous administration of theelin. Moreover, they were similar to the contractions one usually sees in "oestral" rabbits.[3] The waning of the action of theelol was likewise similar to that following administration of theelin, occupying a second 24 hours or more.

Subcutaneous Administration. The rabbits used in this work were intended primarily for drug studies on the effects of certain calcium salts on the uterus. It was desired, therefore, to have "standard" animals which were easily procurable and ready for use within a day or so. Inasmuch as one cannot safely predict the sexual state of intact rabbits unless they happen to be *post partum* and so possibly in heat, we decided to employ recently castrated (3 days to a week or 2) rabbits with uterine fistulae already prepared. The necessity of a large colony and time-consuming breeding was thus obviated. The rabbits so prepared (2-3 kg.) received 25 r.u. of theelol, irrespective of weight, in a single subcutaneous injection on the day preceding an experiment. This was done 16 times with theelol. In only 2 instances did we fail to elicit rhythmic motility in a manner indistinguishable from that observed many times with theelin similarly administered. Upon repetition of the injection in the 2 failures, marked motility was subsequently induced. The failures therefore may probably be explained on the basis of inadequate dosage. Withdrawal of theelol results in quiescence of the fistula in several days. This accords with earlier observations on the effect upon uterine motility of castration at a time when the ovaries contain large follicles.[3]

In conclusion one may say that the 2 isolated oestrogenic substances, tri-hydroxy- and keto-hydroxy-oestrin, exert similar effects upon uterine motility when one employs the uterine fistula technic. Whether this signifies merely similar action or interconversion of one substance into the other *in vivo* is, of course, a matter of conjecture.

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[3] Reynolds, S. R. M., *Am. J. Physiol.*, 1931, **97**, 706.

6841

Action of Pilocarpine and Atropine on Uterine Fistulae in the Unanesthetized Rabbit.

SAMUEL R. M. REYNOLDS. (Introduced by Carl J. Wiggers.)

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The effect of pilocarpine and atropine on the uterine fistula of the unanesthetized rabbit has been observed. The data include observations of 18 injections of pilocarpine and 14 injections of atropine on 5 rabbits over several different days. The method of recording such motility has been described.[1, 2] Adult female rabbits (albino, chinchilla and brown) were castrated at the time of operation. On the day prior to the experiment, 25-50 r.u. of oestrin (Theelin; Theelol from Parke, Davis and Co.) were administered when marked motility was desired.[3] When quiescence was desired, the castrated rabbit was untreated.[3] The effects noted are as follows:

Pilocarpine-HCl. When pre-injection motility is rhythmical in character and of marked amplitude, pilocarpine (0.6-6 mg.) administered intravenously promptly elicits a sustained contraction lasting one to 4 or more minutes. The contractions which follow are frequently of greater duration and increased frequency. The amplitude of these contractions seems not to be regularly affected. When the uterus is relatively quiescent, as recorded by the balloon method, pilocarpine is without effect, or else it elicits a feeble sustained contraction. It is the impression of the observer that when this takes place it occurs in association with other effects of the pilocarpine (cyanosis and pulmonary distress). The non-responsiveness of the quiescent uterine fistula to pilocarpine is similar to its non-responsiveness to posterior pituitary extracts[4] and in anaphylactic shock.[5] Epinephrine, however, does elicit a sustained uterine contraction of the quiescent uterine fistula.

Atropine-sulphate. This drug (1-5 mg.) by itself has no observable effect on either the contracting or quiescent uterine fistula. Its action is limited to (a) preventing a response to subsequent ad-

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[1] Reynolds, S. R. M., *Am. J. Physiol.*, 1930, **92**, 420.

[2] Reynolds, S. R. M., and Friedman, M. H., *Am. J. Physiol.*, 1930, **94**, 696.

[3] Reynolds, S. R. M., *Am. J. Physiol.*, 1931, **97**, 706.

[4] Reynolds, S. R. M., *Am. J. Physiol.*, 1930, **92**, 430.

[5] Weinstein, G. L., Reynolds, S. R. M., and Friedman, M. H., *Am. J. Physiol.*, 1931, **98**, 237.

ministrations of pilocarpine (the duration of this antagonism varied from 9 minutes in one instance to 30 minutes in another); (b) causing relaxation of a uterus already contracted under the influence of pilocarpine. It was repeatedly observed that at the time pilocarpine was inhibited by atropine the uterus responded to epinephrine (0.5-1 cc., 1:50,000) and posterior pituitary extract (0.1-0.5 cc., Parke, Davis).

The present results differ from those which Cushny[6] obtained on anesthetized (paraldehyde) rabbits and cats in that when we observed a feeble sustained contraction of the quiescent uterus following pilocarpine, atropine had no antagonistic action on such a contraction, whereas it did in the experiments described by him. It is suggestive in our experiments, therefore, that the contraction of the quiescent uterus seen by us is only secondarily related to the injection of pilocarpine. Certainly, without concomitant pulmonary distress pilocarpine has not, in our experience, elicited a contraction of the quiescent uterus.

6842

Oxygen and Carbon Dioxide Dissociation Studies on Blood Drawn after Intravenous Injection of Pitressin.*

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In previous communications[1, 2, 3] it was pointed out that certain well defined circulatory and respiratory changes are elicited by the intravenous injection of moderate doses of pituitrin or pitressin into unanesthetized human beings or dogs. For a brief period (5 to 10 minutes) immediately following the administration of the drugs, the venous blood draining the limbs becomes arterial in color and shows a high oxygen content, a rapidly rising lactic acid concentration, and a concomitantly lowered carbon dioxide content. There is at the same time a precipitous decrease in total oxygen consumption and a lowering of the cardiac output. In the immediately ensuing recovery period, which persists for 1½ to 2 hours after the injection, the physiological picture is reversed. An abnormally dark venous blood is found, indicating increased oxygen utilization by the "starved" tissues; a further rise in lactic acid is followed by a gradual return to the pre-injection level; the carbon dioxide content returns slowly to a slightly sub-basal value. Total oxygen consumption and cardiac output show parallel increases above their pre-injection values and then gradually return to the basal level.

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[6] Cushny, A. R., *J. Physiol.*, 1906, **35**, 1.

* This work was aided by a grant from the Hartley Corporation.

[1] Geiling, E. M. K., and DeLawder, A. M., *Bull. Johns Hopkins Hosp.*, 1932, **51**, 1.

[2] Geiling, E. M. K., and DeLawder, A. M., *Bull. Johns Hopkins Hosp.*, 1932, **51**, 335.

[3] Grollman, Arthur, and Geiling, E. M. K., *J. Pharmacol. and Exp. Therap.*, 1932, **46**, 447.

A temporary inability of the blood to give up its oxygen is one cause which might account for the arterial character of the venous blood drawn from the leg 5 to 10 minutes after the intravenous injection of pitressin. To ascertain whether such a factor comes into play, oxygen and carbon dioxide dissociation studies were made on this blood and the results obtained are recorded in the present communication.

Methods. The technique followed was essentially that described by Dill,[4] with minor modifications, the most important of which was the storage of the equilibrated blood in a closed tube over mercury instead of under oil. The carbon dioxide and oxygen contents of the blood were determined by the Van Slyke and Neill method in an apparatus of the portable manometric type, and the gaseous mixtures in the tonometers were analyzed in the Haldane apparatus. The formula used for calculating the gas tensions in the tonometers during equilibration was that recommended by Dill.[4]

Results. Fig. 1 shows the oxygen dissociation curves of normal venous dog's blood, arterial-like venous blood drawn 6 to 8 minutes after the intravenous injection of 0.6 cc. of pitressin, and dark venous blood of the recovery period. It may be seen that the intravenous administration of pitressin to unanesthetized dogs has little effect upon the oxygen dissociation curve of the blood. The only noticeable deviation from the normal is a slight shift of the curve to the right, more marked in the blood obtained during the recovery period. This slight alteration in the pitressinized blood is to be expected from the fact that its lactic acid content is high, being particularly ele-

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[4] Dill, D. B., Appendix to Henderson, L. J., Blood, A Study in General Physiology, Yale University Press, New Haven, 1928.

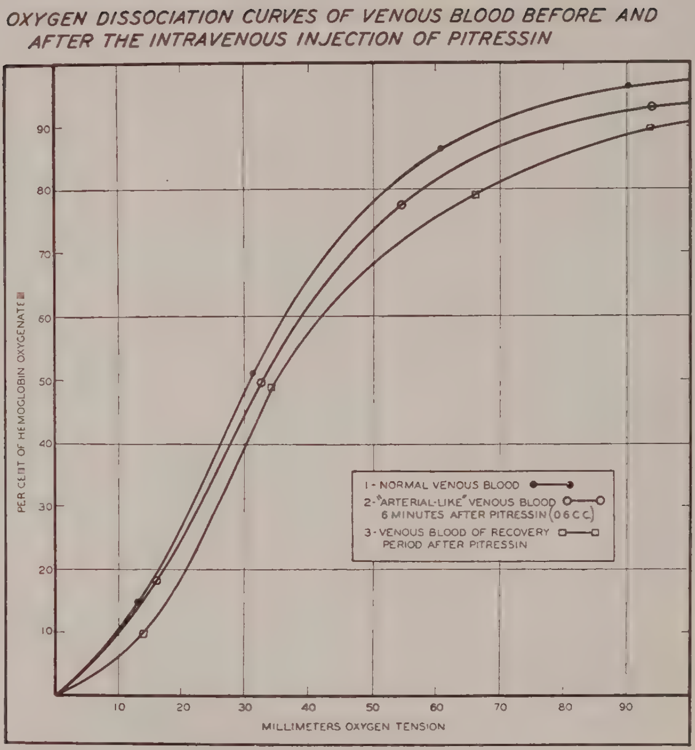

FIG. 1.

vated during the recovery period.[1] Accordingly, any effect of pitressin upon the oxygen dissociation curve of the blood is in the direction of a decreased rather than an increased oxygen affinity. Our curve for normal dog's blood is in close agreement with that reported by Dill, Edwards, Florkin and Campbell.[5]

Results which are essentially similar to those shown in Fig. 1 were obtained when the same procedure was carried out on 2 other unanesthetized dogs.

Fig. 2 represents a comparative study of the carbon dioxide dissociation curves of normal and post-pitressin bloods. The only outstanding variation is a change in the level of the curves of the post-pitressin bloods, with no alteration in the slope. This change, like those noted in the oxygen dissociation studies, is apparently

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[5] Dill, D. B., Edwards, H. T., Florkin, M., and Campbell, R. W., *J. Biol. Chem.*, 1932, **95**, 143.

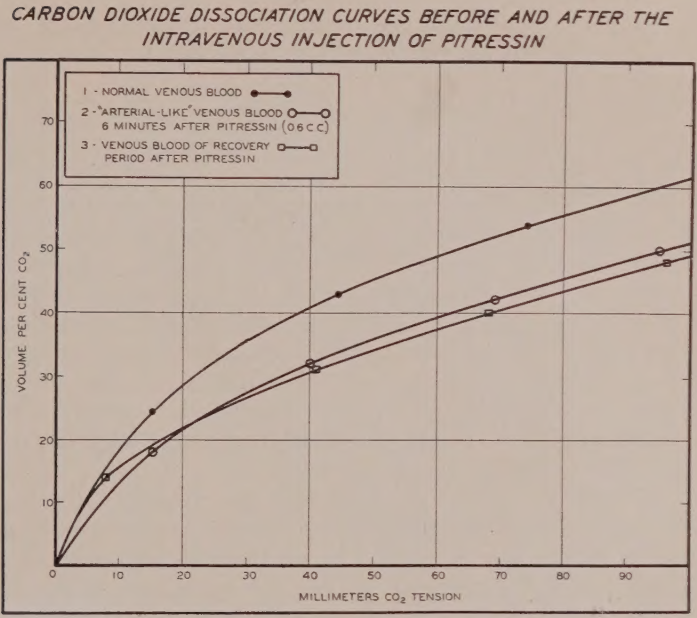

FIG. 2.

due to the presence of lactic acid, which has been shown to increase to a value 3 times the basal level after the injection of pitressin.

From the above findings it seems justifiable to conclude that the high percentage of oxy-hemoglobin in venous blood after the injection of pitressin cannot be attributed to any interference with the power of the blood to yield its oxygen at a given tension.

6843

Effect of Pitressin and Pitocin on Oxygen Consumption of Excised Tissue.

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A direct action of pituitrin and pitressin on the tissue cells so as to render them incapable of taking up oxygen was one of the possible explanations suggested by Geiling and DeLawder for the significant metabolic changes produced in man and animals by these drugs.[1, 2, 3] To test the validity of this suggestion the present work was carried out.

Ahlgren[4] using Thunberg's methylene blue method found that minute concentrations of various posterior pituitary preparations increased tissue respiration. Himwich, Finkelstein and Humphreys,[5] working with the Warburg apparatus, showed on the other hand that the oxygen consumption of excised tissues of white rats is definitely decreased under the influence of pituitrin and pitressin. However, the doses used by them were entirely disproportionate to the amounts generally employed in the intact animal. Accordingly, similar experiments have been carried out with dosages covering a range to include large and small concentrations of post-pituitary preparations (pitressin, pitocin and inactivated pitressin). Even the smallest dose used in this work is relatively large when compared to the effective amount used in the intact dog or in man.

Experimental. White rats weighing approximately 250-300 gm. were used. Liver, heart and diaphragm tissues were excised directly after the animal had been killed by decapitation and their oxygen consumption in the presence of varying quantities of the post-pituitary preparations was immediately determined in Warburg vessels with Barcroft manometers at 37.5°C.[6]

Fresh Ringer-buffer-glucose and saline-buffer-glucose solutions, as recommended by Richardson,[7] were used as nutrient media. A thin slice of tissue not more than 0.5 mm. in thickness was placed in the Warburg vessel with 3 cc. of the medium—Ringer-buffer-glucose solution for liver tissue and saline-buffer-glucose solution for diaphragm and heart (ventricle) tissue. Normal potassium hydroxide (0.2 cc.) was used to absorb the carbon dioxide formed. The post-pituitary extracts, in varying quantities, were added directly to the media in the vessels. Oxygen consumption was measured

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[1] Geiling, E. M. K., and DeLawder, A. M., *Bull. Johns Hopkins Hosp.*, 1932, **51**, 1.

[2] Geiling, E. M. K., and DeLawder, A. M., *Bull. Johns Hopkins Hosp.*, 1932, **51**, 335.

[3] Grollman, Arthur, and Geiling, E. M. K., *J. Pharmacol. and Exp. Therap.*, 1932, **46**, 447.

[4] Ahlgren, G., *Skandin. Arch. f. Physiol.*, 1925, **47** (supplement), 1.

[5] Himwich, H. E., Finkelstein, R., Humphreys, K. E., Proc. Soc. Exp. Biol. and Med., 1931, **29**, 233.

[6] Warburg, O., *Biochem. Z.*, 1923, **142**, 317.

[7] Richardson, H. B., *Physiol. Rev.*, 1929, **9**, 61.

during a period of 2 hours, after which the tissues were dried and weighed.

Pitressin (20 pressor units per cc.) was freed of chloretone before using. A small quantity of a highly purified preparation of pitocin containing 30 oxytocic units per cc. was kindly supplied by Dr. duVigneaud. The pitressin was inactivated by heating with alkali and it was then neutralized with HCl, the NaCl formed being removed by dialysis. An assay showed that 95% of the pressor activity was destroyed.

Results. The accompanying tables show that with a sufficiently small quantity of pitressin the oxygen consumption of excised liver and diaphragmatic tissues is increased, whereas with progressively larger amounts of the drug this effect becomes less pronounced, until finally a sufficiently large dose causes a definite decrease in oxygen consumption. This latter fact is in agreement with the results of Himwich and associates. The results obtained on heart tissue are in the main similar, though less conclusive, except that they show a definite depression of metabolism in the case of the largest amount used. From the data it appears that the various tissues of the body have different threshold levels of activity. Thus the amount of pitressin which suppressed the oxygen consumption of muscle (diaphragm and heart) produced an increase with liver (parenchymatous tissue). With the inactivated pitressin there was a gradual decrease in oxygen consumption as the dosage was increased.

The results with pitocin are inconclusive; but there is a similarity in the results as compared with the inactivated pitressin in the same dosage range.

TABLE I.

O_2 Consumption of Excised Tissues in cu. mm. per mg. per minute.

	Pitressin (A)			Inactivated Pitressin (B)		
Dose	Liver	Diaphragm	Heart (Ventricle)	Liver	Diaphragm	Heart (Ventricle)
Normal	0.0140					
4/50 cc.	0.0082					
Normal	0.0137					
3/50 cc.	0.0134					
Normal	0.0092	0.0144	0.0119	0.0102	0.0129	0.0177
2/50 cc.	0.0132	0.0106	0.0091	0.0065	0.0053	0.0065
Normal	0.0095	0.0102	0.0167	0.0102	0.0129	0.0177
1/50 cc.	0.0127	0.0085	0.0165	0.0075	0.0062	0.0093
Normal		0.0085	0.0078		0.0129	0.0177
1/100 cc.		0.0106	0.0084		0.0095	0.0138

TABLE II.

O_2 Consumption of Excised Tissues in cu. mm. per mg. per minute.

	Pitocin.		
Doses	Liver	Diaphragm	Heart (Ventricle)
Normal	0.0079	0.0071	0.0089
2/50 cc.	0.0063	0.0057	0.0058
1/50 cc.	0.0057	0.0058	0.0071
1/100 cc.	0.0061	0.0072	0.0070

One hesitates to place any definite interpretation on the effects produced on isolated tissues by the larger doses of pitressin, especially since the inactivated material similarly reduces the oxygen consumption. Of course, alkali treatment may do more than merely render inactive the small amount of hormone present in the pitressin fraction, which is a mixture of organic substances. There may be formed new products which depress the metabolism of the tissues. Furthermore the tissues of the rat may be much less reactive to posterior pituitary preparations than are those of human beings or dogs. Hence it may be that excised human or dog tissues under similar conditions would show depression of respiration with very much smaller dosages. On the other hand, the data presented show clearly that there is an increase in oxygen consumption of excised tissues with the smaller concentrations of pitressin. It would thus seem that the initial lowering of metabolism produced by relatively small doses of the drug when injected into the intact animal does not find an explanation in the experiments here reported.